(54) Title: 11-BETA-HYDROXYSTEROID DEHYDROGENASE 1 INHIBITORS USEFUL FOR THE TREATMENT OF DIABETES, OBESITY AND DYSLIPIDEMIA

$$R^1X \underset{N-N}{\overset{\overset{\displaystyle WR^2}{\underset{|}{N}}}{\bigwedge}} ZR^3 \quad (1)$$
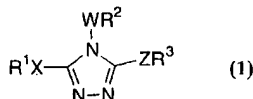
(57) Abstract: Compounds having Formula (I), including pharmaceutically acceptable salts and prodrugs thereof: are selective inhibitors of the 11β-HSD1 enzyme. They inhibit the 11β-HSD1-mediated conversion of cortisone and other 11-keto-glucocorticoids to cortisol and other 11β-hydroxy-glucocorticoids. The 11β-HSD1 inhibitors therefore decrease the amount of cortisol in target tissues, thereby modulating the effects of cortisol. Modulation of cortisol may be effective in controlling non-insulin-dependent diabetes (NIDDM), hyperglycemia, obesity, insulin resistance, dyslipidemia, hyperlipidemia, hypertension, Syndrome X, and other symptoms associated with NIDDM or with excess cortisol in the body.

WO 03/065983 A2

TITLE OF THE INVENTION

11-BETA-HYDROXYSTEROID DEHYDROGENASE 1 INHIBITORS USEFUL FOR THE TREATMENT OF DIABETES, OBESITY AND DYSLIPIDEMIA

5 FIELD OF THE INVENTION

The instant invention is concerned with inhibitors of the 11-beta-hydroxysteroid dehydrogenase Type I enzyme, including pharmaceutically acceptable salts and prodrugs thereof, which are useful as therapeutic compounds, particularly in the treatment of non-insulin dependent type 2 diabetes mellitus (NIDDM), insulin

10 resistance, obesity, lipid disorders, and other diseases and conditions that are mediated by excess cortisol.

BACKGROUND OF THE INVENTION

Diabetes is a disease derived from multiple causative factors and

15 characterized by elevated levels of plasma glucose (hyperglycemia) in the fasting state or after administration of glucose during an oral glucose tolerance test. There are two generally recognized forms of diabetes. In type 1 diabetes, or insulin-dependent diabetes mellitus (IDDM), patients produce little or no insulin, the hormone which regulates glucose utilization. In type 2 diabetes, or noninsulin-dependent diabetes

20 mellitus (NIDDM), insulin is still produced in the body. Patients often have hyperinsulinemia (plasma insulin levels that are the same or even elevated in comparison with non-diabetic subjects); however, these patients have developed insulin resistance, which is a resistance to the effect of insulin in stimulating glucose and lipid metabolism in the main insulin-sensitive tissues, which are muscle, liver and

25 adipose tissues. Patients who are insulin resistant but not diabetic have elevated insulin levels that compensate for the insulin resistance so that serum glucose levels are not elevated. In patients with NIDDM, the plasma insulin levels, even when they are elevated, are insufficient to overcome the pronounced insulin resistance.

Insulin resistance is not primarily due to a diminished number of

30 insulin receptors but to a post-insulin receptor binding defect that is not yet completely understood. This resistance to insulin responsiveness results in insufficient insulin activation of glucose uptake, oxidation and storage in muscle and inadequate insulin repression of lipolysis in adipose tissue and of glucose production and secretion in the liver.

Persistent or uncontrolled hyperglycemia that occurs with diabetes is associated with increased and premature morbidity and mortality. Often abnormal glucose homeostasis is associated both directly and indirectly with obesity, hypertension, and alterations of the lipid, lipoprotein and apolipoprotein metabolism
5   and other metabolic and hemodynamic disease. Therefore patients with type 2 diabetes mellitus are at an especially increased risk of macrovascular and microvascular complications, including atherosclerosis, coronary heart disease, stroke, peripheral vascular disease, hypertension, nephropathy, neuropathy, and retinopathy. Therefore, therapeutic control of glucose homeostasis, lipid metabolism, obesity, and
10   hypertension are critically important in the clinical management and treatment of diabetes mellitus.

Many patients who have insulin resistance but have not developed type 2 diabetes are at a risk of developing at least several symptoms selected from a group of symptoms that are often referred to as syndrome X, or the metabolic syndrome.
15   This syndrome is characterized by insulin resistance, abdominal obesity, hyperinsulinemia, high blood pressure, low HDL, and high VLDL. These patients, whether or not they develop overt diabetes mellitus, are at increased risk of the macrovascular and microvascular complications of type 2 diabetes listed above (e.g. atherosclerosis and coronary heart disease).

20   Insulin resistance is not primarily due to a diminished number of insulin receptors but to a post-insulin receptor binding defect that is not yet completely understood. This resistance to insulin responsiveness results in insufficient insulin activation of glucose uptake, oxidation and storage in muscle and inadequate insulin repression of lipolysis in adipose tissue and of glucose production
25   and secretion in the liver.

The available treatments for type 2 diabetes have not changed substantially in many years, and these treatments have recognized limitations. Physical exercise and reductions in dietary intake of calories often dramatically improve the diabetic condition, but compliance with this treatment is very poor
30   because of well-entrenched sedentary lifestyles and excess food consumption, especially of foods containing high amounts of saturated fat. Increasing the plasma level of insulin by administration of sulfonylureas (e.g. tolbutamide and glipizide) or meglitinide, which stimulate the pancreatic β–cells to secrete more insulin, and/or by injection of insulin when sulfonylureas or meglitinide become ineffective, can result
35   in insulin concentrations high enough to stimulate the very insulin-resistant tissues.

However, dangerously low levels of plasma glucose can result from administration of insulin or insulin secretagogues (sulfonylureas or meglitinide), and an increased level of insulin resistance due to the even higher plasma insulin levels can occur. The biguanides increase insulin sensitivity resulting in some correction of hyperglycemia.

5 However, the two biguanides, phenformin and metformin, can induce lactic acidosis and nausea/diarrhea. Metformin has fewer side effects than phenformin and is often prescribed for the treatment of Type 2 diabetes.

The glitazones (i.e. 5-benzylthiazolidine-2,4-diones) are a newer class of compounds with the potential for ameliorating hyperglycemia and other symptoms

10 of type 2 diabetes. These agents substantially increase insulin sensitivity in muscle, liver and adipose tissue in several animal models of type 2 diabetes, resulting in partial or complete correction of the elevated plasma levels of glucose without occurrence of hypoglycemia. The glitazones that are currently marketed are agonists of the peroxisome proliferator activated receptor (PPAR) gamma subtype. PPAR-

15 gamma agonism is generally believed to be responsible for the improved insulin sensititization that is observed with the glitazones. Newer PPAR agonists that are being developed for treatment of Type 2 diabetes and/or dyslipidemia are agonists of one or more of the PPAR alpha, gamma and delta subtypes.

There is a continuing need for new methods of treating the disease.

20 New biochemical approaches that have been recently introduced or are under active development include treatment with alpha-glucosidase inhibitors (e.g. acarbose), protein tyrosine phosphatase-1B (PTP-1B) inhibitors, and inhibitors of the dipeptidyl peptidase-IV (DPP-IV) enzyme. Inhibition of the expression of PTP-1B by the use of antisense oligonucleotides is also under investigation.

25 Another method of treating type 2 diabetes that has been suggested in the literature is the use of inhibitors of the 11-ß-hydroxysteroid dehydrogenase type 1 enzyme (11β-HSD1) to reduce the amount of active glucocorticoids in tissues where glucose is metabolized. See J. R. Seckl et al., Endocrinology, 142: 1371-1376, 2001, and references cited therein. There are so far only a few reports of compounds that

30 are inhibitors of the 11β-HSD1 enzyme.

SUMMARY OF THE INVENTION

A class of compounds is disclosed that inhibits the 11β-HSD1 enzyme, thereby inhibiting the reduction of cortisone and other 11-keto steroids to cortisol and

35 other 11β-hydroxysteroids. Administration of the compounds decreases the level of

cortisol and other 11β-hydroxysteroids in target tissues, thereby reducing the effects of excessive amounts of cortisol and other 11β-hydroxysteroids. Inhibition of 11β-HSD1 can be used to treat and control diseases mediated by abnormally high levels of cortisol and other 11β-hydroxysteroids, such as NIDDM, obesity, hypertension, and

5   dyslipidemia.

The compounds of the present invention have the structure shown in formula I below, or a pharmaceutically acceptable salt or prodrug thereof:

$$R^1X \overset{\displaystyle \overset{WR^2}{\underset{|}{N}}}{\underset{N-N}{\diagup\!\!\diagup\!\!\diagdown}} ZR^3$$

(I)
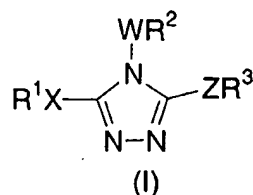
In formula I:

10   R^1 is adamantyl, unsubstituted or substituted with one to five substituents independently selected from halogen, OCH_3, OCF_3, CH_3, CF_3, and phenyl, wherein said phenyl is unsubstituted or substituted with one to three halogens;

W is selected from the group consisting of NR^a and a single bond;

15   X is selected from the group consisting of CH_2 and a single bond;

Z is selected from the group consisting of S and a single bond;

R^a is selected from the group consisting of hydrogen and C_{1-6} alkyl, wherein alkyl is unsubstituted or substituted with one to five fluorines;

20

R^2 is selected from the group consisting of

      hydrogen,

      C_{1-10} alkyl, unsubstituted or substituted with one to six substituents

independently selected from zero to five halogens and zero or one group selected from

25   hydroxy and C_{1-3} alkoxy, said alkoxy group being unsubstituted or substituted with

one to three halogens,

      C_{2-10} alkenyl, unsubstituted or substituted with one to six substituents

independently selected from zero to five halogens and zero or one group selected from

hydroxy and C_{1-3} alkoxy, said alkoxy group being unsubstituted or substituted with

30   one to three halogens,

CH_2CO_2H,

$CH_2CO_2C_{1-6}$ alkyl,

CH_2CONHR^a,

$(CH_2)_{0-2}C_{3-9}$ cycloalkyl,

$(CH_2)_{0-2}C_{5-12}$ bicycloalkyl,

$(CH_2)_{0-2}$adamantyl, and

$(CH_2)_{0-2}R$;

wherein said C_{3-9} cycloalkyl and C_{5-12} bicycloalkyl optionally have one to two double bonds, and said C_{3-9} cycloalkyl, C_{5-12} bicycloalkyl, and adamantyl are unsubstituted or substituted with one to six substituents independently selected from (a) zero to five halogens, CH_3, CF_3, OCH_3, and OCF_3, and (b) zero or one phenyl, said phenyl being unsubstituted or substituted with one to four groups independently selected from halogen, OCH_3, OCF_3, CH_3, and CF_3;
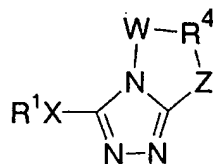
R^3 is selected from the group consisting of

hydrogen,

C_{1-10} alkyl, unsubstituted or substituted with one to six substituents independently selected from zero to five halogens and zero or one group selected from hydroxy and C_{1-3} alkoxy, said alkoxy group being unsubstituted or substituted with one to three halogens,

C_{2-10} alkenyl, unsubstituted or substituted with one to six substituents independently selected from zero to five halogens and zero or one group selected from hydroxy and C_{1-3} alkoxy, said alkoxy group being unsubstituted or substituted with one to three halogens,

YC_{3-9} cycloalkyl,

YC_{5-12} bicycloalkyl,

Yadamantyl, and

YR;

wherein said C_{3-9} cycloalkyl and C_{5-12} bicycloalkyl optionally have one to two double bonds, and said C_{3-9} cycloalkyl, C_{5-12} bicycloalkyl, and adamantyl are unsubstituted or substituted with one to six substituents independently selected from (a) zero to five halogens, CH_3, CF_3, OCH_3, and OCF_3, and (b) zero or one phenyl, said phenyl being unsubstituted or substituted with one to four groups independently selected from halogen, OCH_3, OCF_3, CH_3, and CF_3;

R is selected from the group consisting of benzodioxolane, furan, tetrahydrofuran, thiophene, tetrahydrothiophene, dihydropyran, tetrahydropyran, pyridine, piperidine, benzofuran, dihydrobenzofuran, benzothiophene, dihydrobenzothiophene, indole, dihydroindole, indene, indane, 1,3-dioxolane, 1,3-dioxane, phenyl, and naphthyl; wherein R is unsubstituted or substituted with one to four groups independently selected from halogen, C_{1-4} alkylthio, C_{1-4} alkylsulfinyl, C_{1-4} alkylsulfonyl, C_{2-4} alkenylsulfonyl, CN, OH, OCH_3, OCF_3, and C_{1-4} alkyl, said C_{1-4} alkyl being unsubstituted or substituted with one to five halogens or one substituent selected from OH and C_{1-3} alkoxy; and

Y is selected from $(CH_2)_{0-2}$ and $(-HC=CH-)$;

or alternatively R^2 and R^3 taken together form a bridging group R^4, providing a compound of structural formula Ia:

$$
\begin{array}{c}
W-R^4 \\
| \quad \backslash \\
N \quad Z \\
R^1X-\underset{\underset{N-N}{\vee}}{\vee}
\end{array}
$$

Ia

wherein R^4 is

a C_{2-8} alkylene group, optionally containing one heteroatom selected from O and NR^b between two adjacent carbon atoms of said C_{2-8} alkylene group, optionally containing one to two carbon-carbon double bonds when R^4 is a C_{3-8} alkylene group, and optionally also comprising a carbon-carbon single bond connecting two non-adjacent carbon atoms of said C_{2-8} alkylene group, or

a C_{4-8} cycloalkyl group;

wherein R^b is selected from the group consisting of hydrogen and C_{1-6} alkyl, unsubstituted or substituted with one to six substituents independently selected from zero to five fluorines and zero or one phenyl, said phenyl being unsubstituted or substituted with one to three substituents independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3;

wherein R^4 is unsubstituted or substituted with one to five R^c substituents, wherein each R^c is independently selected from halogen, OH, OCH_3, OCF_3, C_{1-6} alkyl, C_{2-6} alkenyl, phenyl, biphenyl, C_{3-8} cycloalkyl, C_{1-6} alkyloxycarbonyl, an epoxide group bridging 2 adjacent carbons, and 1,3-dioxolanyl geminally disubstituted onto one carbon of R^4, wherein each C_{1-6} alkyl and C_{2-6} alkenyl is unsubstituted or substituted with one to five substituents independently selected from zero to three halogens and zero to two groups selected from phenyl, C_{1-6} alkyloxycarbonyl, 1,3-dioxolanyl geminally disubstituted onto one carbon, and CN, and wherein each phenyl, biphenyl, and C_{3-8} cycloalkyl, either as R^c or as a substituent on R^c, is unsubstituted or substituted with one to three groups independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3;

wherein R^4 optionally has a fused phenyl ring, a benzodioxinyl ring, or a dihydrobenzodioxinyl ring, said phenyl ring, benzodioxinyl ring, and dihydrobenzodioxinyl ring being unsubstituted or substituted with one to three substituents independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3; and

wherein R^4, including said optional fused phenyl ring, benzodioxinyl ring, or dihydrobenzodioxinyl ring and including all substituents on R^4 and said fused phenyl ring, benzodioxinyl ring, or dihydrobenzodioxinyl ring, has no more than 20 carbon atoms;

with the provisos that

(a) when X and W represent single bonds, Z is sulfur, R^1 is unsubstituted adamantyl, and R^3 is hydrogen, then R^2 is not hydrogen, methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, 2-butyl, tert-butyl, phenyl, CH_2phenyl, or cyclohexyl;

(b) when X and W represent single bonds, Z is sulfur, R^1 is unsubstituted adamantyl, and R^3 is ethyl, 3-propenyl, CH_2phenyl, 4-Cl-CH_2phenyl, or 4-NO_2-CH_2phenyl, then R^2 is not methyl;

(c) when X and W represent single bonds, Z is sulfur, R^1 is unsubstituted adamantyl, and R^3 is CH_2-(CO)-4-F-phenyl, then R^2 is not phenyl;

(d) when X and Z represent single bonds and R^1 is unsubstituted adamantyl, then R^2 and R^3 taken together cannnot form a C_{3-5} alkylene R^4 bridging group; and

(e) R^2 and R^3 are not both hydrogen.

DETAILED DESCRIPTION OF THE INVENTION

The compounds of structural formula I of the present invention have numerous embodiments, which are described below.

$$R^1X \overset{\overset{\displaystyle WR^2}{\displaystyle |}}{\underset{N-N}{\overset{N}{\diagup\diagdown}}} ZR^3$$

(I)
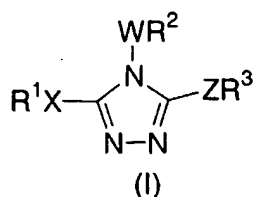
One embodiment comprises compounds having formula I as described above, where R^2 and R^3 are substituent groups but are not taken together to form a bridging group R^4 to provide a compound having formula Ia,

$$R^1X \overset{\overset{\displaystyle W-R^4}{\displaystyle |}}{\underset{N-N}{\overset{N}{\diagup\diagdown}}} Z$$

(Ia)
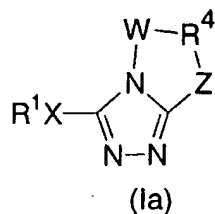
Another embodiment comprises compounds all of which have formula Ia as described above, but does not include compounds that have formula I.

Another embodiment comprises compounds having formula I as described above, wherein

R^1 is adamantyl, unsubstituted or substituted with one to five substituents independently selected from halogen, OCH_3, OCF_3, CH_3, CF_3, and phenyl, wherein said phenyl is unsubstituted or substituted with one to three halogens;

X, W, and Z are single bonds;

R^2 is selected from the group consisting of
hydrogen,
C_{1-6} alkyl, unsubstituted or substituted with one to four substituents

-8-

independently selected from zero to three halogens and zero or one group selected from hydroxy and C_{1-3} alkoxy, said alkoxy group being unsubstituted or substituted with one to three halogens,

   C_{2-4} alkenyl, unsubstituted or substituted with one to four substituents

5 independently selected from zero to three halogens and zero or one group selected from hydroxy and C_{1-3} alkoxy, said alkoxy group being unsubstituted or substituted with one to three halogens,

   CH_2CO_2H,

   $CH_2CO_2C_{1-3}$ alkyl,

10   CH_2CONHR^a,

   $(CH_2)_{0-1}C_{3-6}$ cycloalkyl,

   $(CH_2)_{0-1}C_{4-6}$ cycloalkenyl,

   $(CH_2)_{0-1}$phenyl,

   $(CH_2)_{0-1}$furyl,

15 wherein cycloalkyl, cycloalkenyl, phenyl, and furyl are unsubstituted or substituted with one to three groups independently selected from halogen, OCH_3, OCF_3, CH_3, and CF_3;

R^a is selected from the group consisting of hydrogen and C_{1-6} alkyl, wherein alkyl is

20 unsubstituted or substituted with one to five fluorines; and

R^3 is selected from the group consisting of

   hydrogen,

   C_{1-6} alkyl, unsubstituted or substituted with one to five halogens,

25   C_{2-6} alkenyl, unsubstituted or substituted with one to five halogens,

   $CH_2)_{0-1}C_{3-6}$ cycloalkyl, wherein cycloalkyl has one double bond and is

unsubstituted or substituted with one to five substituents independently selected from the group consisting of (a) zero to five halogens and methyl and (b) zero or 1 phenyl,

   $(CH_2)_{0-1}$ adamantyl, unsubstituted or substituted with one to four substituents

30 independently selected from halogen and methyl,

   $(CH_2)_{0-1}$ phenyl, unsubstituted or substituted with one to three substituents independently selected from methyl, cyano, hydroxymethyl, CF_3, OCF_3, hydroxy, OCH_3, halogen and $S(O)_{0-2}CH_3$, and

   YR, wherein Y is selected from the group consisting of CH_2, (-HC=CH-), and

35 a bond, and R is selected from the group consisting of benzodioxolane, furan,

thiophene, dihydrobenzofuran, tetrahydrofuran, tetrahydropyran, and indane, wherein R is unsubstituted or substituted with one to three halogens.

Another embodiment of compounds of the present invention comprises compounds that have formula I but not formula Ia as described above, wherein

5   R^1 is adamantyl, unsubstituted or substituted with one to five substituents independently selected from halogen, OCH_3, OCF_3, CH_3, CF_3, and phenyl, wherein said phenyl is unsubstituted or substituted with one to three halogens;

X is a single bond;

10   Z is S;

WR^2 is selected from the group consisting of
        NH_2,
        hydrogen,
15   C_{1-6} alkyl, unsubstituted or substituted with one to four substituents independently selected from zero to three halogens and zero or one group selected from hydroxy and methoxy,
        C_{2-4} alkenyl, unsubstituted or substituted with one to three halogens,
        $(CH_2)_{0-1}C_{3-6}$ cycloalkyl, and
20      $(CH_2)_{0-2}R$, wherein R is selected from the group conssiting of phenyl, furan, tetrahydrofuran, and piperidine; wherein R and cycloalkyl are unsubstituted or substituted with one to three groups independently selected from halogen, OCH_3, OCF_3, CH_3, and CF_3; and

25   R^3 is selected from the group consisting of
        hydrogen,
        C_{1-6} alkyl, unsubstituted or substituted with hydroxy, methoxy, or one to five halogens,
        C_{2-6} alkenyl, unsubstituted or substituted with hydroxy, methoxy, or one to
30   five halogens,
        $(CH_2)_{0-2}C_{3-8}$ cycloalkyl, wherein cycloalkyl has one double bond and is unsubstituted or substituted with one to four substituents independently selected from the group consisting of (a) zero to three halogens and methyl and (b) zero or 1 phenyl, and

$(CH_2)_{0-1}R$, wherein R is selected from the group consisting of 1,3-dioxolane, 1,3-dioxane, phenyl, furan, and pyridine; wherein R is unsubstituted or substituted with one to three groups independently selected from halogen, OCH_3, OCF_3, CH_3, and CF_3.

5   Another embodiment comprises compounds that have formula Ia as described above wherein

R^1 is adamantyl, unsubstituted or substituted with one to five substituents independently selected from halogen, OCH_3, OCF_3, CH_3, CF_3, and phenyl, wherein said phenyl is unsubstituted or substituted with one to three halogens;

10   X is a bond;

Z is S;

W is a bond or NH; and

R^4 is a C_{2-8} alkylene group, unsubstituted or substituted with one to three substituents R^c, where each R^c is independently selected from halogen, CH_3, CF_3,

15   and phenyl, wherein phenyl is unsubstituted or substituted with one to three substituents independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3.

Another embodiment relates to compounds of formula Ia, as described below, or a pharmaceutically acceptable salt or prodrug thereof, wherein:

R^1 is adamantyl, unsubstituted or substituted with one to five substituents

20   independently selected from halogen, OCH_3, OCF_3, CH_3, CF_3, and phenyl, wherein said phenyl is unsubstituted or substituted with one to three halogens;

X is selected from the group consisting of CH_2 and a single bond;

W and Z are single bonds; and

25

R^4 is

        a C_{3-8} alkylene group, optionally containing one heteroatom selected from O and NR^b between two adjacent carbon atoms of said C_{3-8} alkylene group, optionally containing one to two carbon-carbon double bonds when R^4 is a C_{3-8} alkylene group,

30   and optionally also comprising a carbon-carbon single bond connecting two non-adjacent carbon atoms of said C_{3-8} alkylene group, or

        a C_{4-8} cycloalkyl group;

wherein R^b is selected from the group consisting of hydrogen and C_{1-6} alkyl, unsubstituted or substituted with one to six substituents independently selected from

zero to five fluorines and zero to one phenyl, said phenyl being unsubstituted or substituted with one to three substituents independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3;

wherein R^4 is unsubstituted or substituted with one to five R^c substituents, wherein

5   each R^c is independently selected from halogen, OH, OCH_3, OCF_3, C_{1-6} alkyl, C_{2-6} alkenyl, phenyl, biphenyl, C_{3-8} cycloalkyl, C_{1-6} alkyloxycarbonyl, an epoxide group bridging 2 adjacent carbons, and 1,3-dioxolanyl geminally disubstituted onto one carbon of R^4, wherein each C_{1-6} alkyl and C_{2-6} alkenyl is unsubstituted or substituted with one to five substituents independently selected from zero to three

10   halogens and zero to two groups selected from phenyl, C_{1-6} alkyloxycarbonyl, 1,3-dioxolanyl geminally disubstituted onto one carbon, and CN, and wherein each phenyl, biphenyl, and C_{3-8} cycloalkyl, either as R^c or as a substituent on R^c, is unsubstituted or substituted with one to three groups independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3;

15

wherein R^4 optionally has a fused phenyl ring, a benzodioxinyl ring, or a dihydrobenzodioxinyl ring, said phenyl ring, benzodioxinyl ring, and dihydrobenzodioxinyl ring being unsubstituted or substituted with one to three substituents independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3; and

20

wherein R^4, including said optional fused phenyl ring, benzodioxinyl ring, or dihydrobenzodioxinyl ring and including all substituents on R^4 and said fused phenyl ring, benzodioxinyl ring, or dihydrobenzodioxinyl ring, has no more than 20 carbon atoms.

25   Another embodiment of compounds having formula I or formula Ia as described above, comprises compounds in which Z is S and WR^2 is selected from NH_2 and R^2.

Another subset of compound having formula I or formula Ia as described above includes compound in which W and Z are single bonds.

30   Illustrative, but nonlimiting, examples of compounds of the present invention that are useful as inhibitors of the 11-beta-hydroxysteroid dehydrogenase Type I enzyme are the following:

5
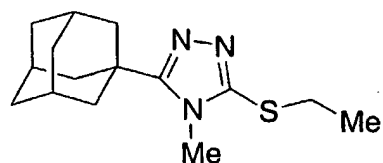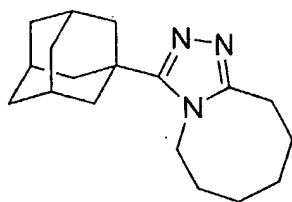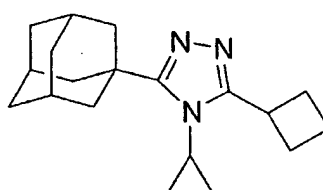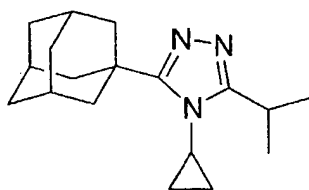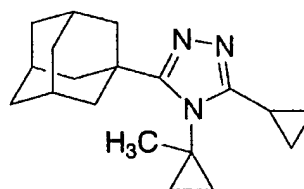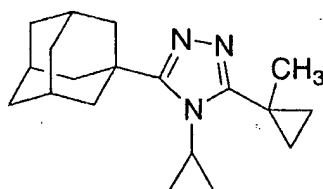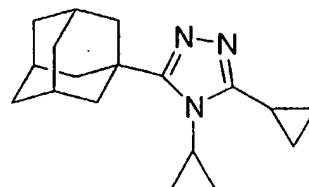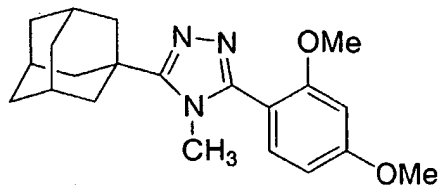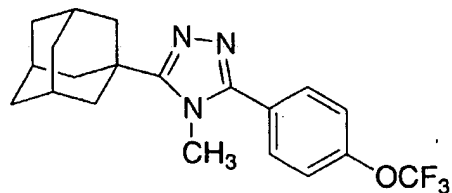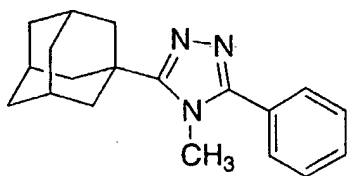
or a pharmaceutically acceptable salt or prodrug thereof.

<u>Definitions:</u>

"Ac" is acetyl, which is CH3C(O)-.

"Alkyl", as well as other groups having the prefix "alk", such as alkoxy

10　or alkanoyl, means carbon chains which may be linear or branched or combinations

thereof, unless the carbon chain is defined otherwise.　Examples of alkyl groups

include methyl, ethyl, propyl, isopropyl, butyl, sec- and tert-butyl, pentyl, hexyl, heptyl, octyl, nonyl, and the like.

"Alkenyl" means carbon chains which contain at least one carbon-carbon double bond, and which may be linear or branched or combinations thereof,
5   unless the carbon chain is defined otherwise. Examples of alkenyl include vinyl, allyl, isopropenyl, pentenyl, hexenyl, heptenyl, 1-propenyl, 2-butenyl, 2-methyl-2-butenyl, and the like.

"Alkynyl" means carbon chains which contain at least one carbon-carbon triple bond, and which may be linear or branched or combinations thereof.
10 Examples of alkynyl include ethynyl, propargyl, 3-methyl-1-pentynyl, 2-heptynyl and the like.

"Alkylene" refers to carbon chains that are bifunctional, such as $-CH_2-$, $-(CH_2)_2-$, $-(CH_2)_3-$, and the like. Alkylene groups are linear or branched, unless otherwise indicated. For comparison, alkyl groups are monofunctional.
15 "Cycloalkyl" means a saturated carbocyclic ring having a specified number of carbon atoms. Examples of cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and the like. A cycloalkyl group generally is monocyclic unless stated otherwise. Bicycloalkyl and tricycloalkyl are bicyclic and tricyclic carbocyclic ring systems. Cycloalkyl, bicycloalkyl and tricycloalkyl groups
20 are saturated unless otherwise defined.

"Aryl" means a mono- or polycyclic aromatic ring system containing only carbon ring atoms. The preferred aryls are monocyclic or bicyclic 6-10 membered aromatic ring systems. Phenyl and naphthyl are preferred aryls. The most preferred aryl is phenyl.
25 "Heterocycle" means a saturated or unsaturated ring (including aromatic rings) containing at least one heteroatom selected from N, S and O (including SO and SO_2). Examples of heterocycles include tetrahydrofuran, piperidine, piperazine, morpholine, thiomorpholine, and tetrahydrothiophene 1,1-dioxide.
30 "Heteroaryl" means an aromatic heterocycle that contains at least one ring heteroatom selected from N, O and S (including SO and SO_2). Heteroaryls can be fused to other heteroaryls or to other kinds of rings, such as aryls, cycloalkyls and heterocycles that are not aromatic. Examples of monocyclic heteroaryl substituents include pyrrolyl, isoxazolyl, isothiazolyl, pyrazolyl, pyridyl, oxazolyl, oxadiazolyl,
35 thiadiazolyl, thiazolyl, imidazolyl, triazolyl, tetrazolyl, furanyl, triazinyl, thienyl, and

pyrimidyl. Examples of ring systems in which a heteroaryl shares a common side with phenyl include benzisoxazole, benzoxazole, benzothiazole, benzimidazole, benzofuran, benzothiophene (including S-oxide and dioxide), quinoline, indole, isoquinoline, dibenzofuran, and the like. Heteroaromatic rings can also be fused

5   together, as in furo(2,3-b)pyridyl, for example.

"Halogen" includes fluorine, chlorine, bromine and iodine. Chlorine and fluorine are generally preferred. Fluorine is most often preferred when the halogens are substituted on an alkyl or alkoxy group (e.g. CF_3O and CF_3CH_2O).

The term "composition," as in pharmaceutical composition, is intended

10   to encompass a product comprising the active ingredient(s) and the inert ingredient(s) that make up the carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical

15   compositions of the present invention encompass compositions made by admixing a compound of the present invention and a pharmaceutically acceptable carrier.

Optical Isomers - Diastereomers - Geometric Isomers – Tautomers:

Compounds of Formula I and Formula Ia may contain one or more

20   asymmetric centers and can thus occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual diastereomers. The present invention is meant to comprehend all such isomeric forms of the compounds of Formula I.

Some of the compounds described herein contain olefinic double

25   bonds, and unless specified otherwise, are meant to include both E and Z geometric isomers.

Some of the compounds described herein may exist as tautomers, which have different points of attachment of hydrogen accompanied by one or more double bond shifts. For example, a ketone and its enol form are keto-enol tautomers.

30   The individual tautomers as well as mixtures thereof are encompassed with compounds of Formula I and Formula Ia. In the current application, thiol substituents on the carbon of the triazole ring have thioketone tautomers, and the thioketone tautomer is also represented by the formula showing the triazole with a thiol group on the ring.

If desired, racemic mixtures of compounds of Formula I and Formula Ia may be separated so that the individual enantiomers are isolated. The separation can be carried out by methods well known in the art, such as the coupling of a racemic mixture of compounds of Formula I or Formula Ia to an enantiomerically pure

5   compound to form a diastereomeric mixture, which is then separated into individual diastereomers by standard methods, such as fractional crystallization or chromatography. The coupling reaction is often the formation of salts using an enantiomerically pure acid or base. The diasteromeric derivatives may then be converted to the pure enantiomers by cleaving the added chiral residue from the

10 diastereomeric compound. The racemic mixture of the compounds of Formula I or Formula Ia can also be separated directly by chromatographic methods utilizing chiral stationary phases, which methods are well known in the art.

Alternatively, enantiomers of compounds of the general Formula I and Formula Ia may be obtained by stereoselective synthesis using optically pure starting materials or reagents of known configuration. Such methods are well known in the

15 art.

Compounds of Formula I and Ia may have more than one asymmetric center. Such compounds may occur as mixtures of diasteromers, which can be separated into individual diasteromers by standard methods, and the diastereomers can

20 be further separated to individual enantiomers as described above.

Salts:

The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or

25 organic bases and inorganic or organic acids. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium, and sodium salts. Salts in the solid form may exist in more than one crystal structure, and may also be in the

30 form of hydrates. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-

35 dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethyl-morpholine, N-

-16-

ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like.

5   When the compound of the present invention is basic, salts may be prepared from pharmaceutically acceptable non-toxic acids, including inorganic and organic acids. Such acids include acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethanesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, 10   pantothenic, phosphoric, succinic, sulfuric, tartaric, p-toluenesulfonic acid, and the like. Particularly preferred pharamaceutically acceptable acids include citric, hydrobromic, hydrochloric, maleic, phosphoric, sulfuric, and tartaric acids. In most cases, compounds of the present invention are basic because the triazole ring is basic. The triazole compounds of this invention may also be made and handled as non- 15   pharmaceutically acceptable salts (e.g. trifluoroacetate salts) during synthesis before they are used in making pharmaceuticals.

It will be understood that, as used herein, references to the compounds of Formula I and Formula Ia are meant to also include the pharmaceutically acceptable salts, and also salts that are not pharmaceutically acceptable when they are used as 20   precursors to the free compounds or their pharmaceutically acceptable salts or in other synthetic manipulations.

Metabolites – Prodrugs:

Metabolites of the compounds of this invention that are therapeutically 25   active and that are also defined by Formula I are also within the scope of this invention. Prodrugs are compounds that are converted to therapeutically active compounds as they are being administered to a patient or after they have been administered to a patient. Prodrugs, which themselves do not have the structures claimed herein, but which are converted to active compounds defined by Formula I 30   during or after administration to a mammalian patient, are prodrugs and are compounds of this invention, as are their active metabolites that are defined by Formula I.
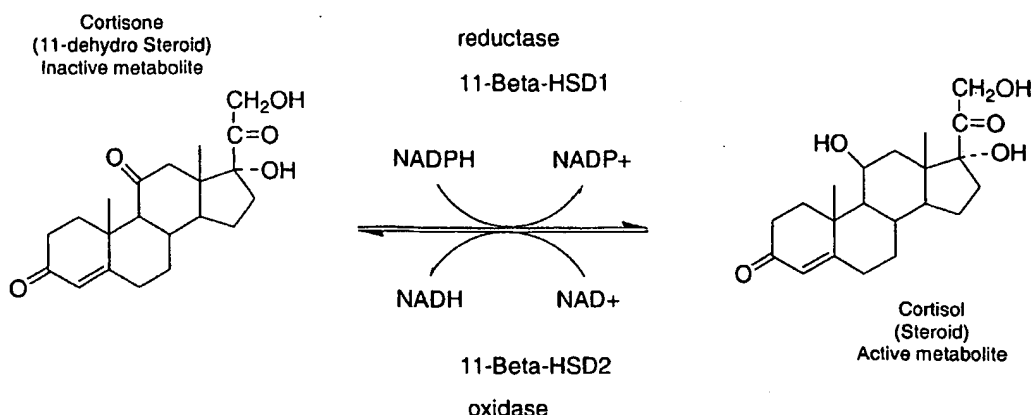
Biochemical Mechanism:

The compounds of this invention are selective inhibitors of the
11β-HSD1 enzyme. Their utility in treating type 2 diabetes, high blood pressure,
5   dyslipidemia, obesity, and other diseases and conditions is believed to derive from the
biochemical mechanism described below. This mechanism is provided for clarification
only, and is non-limiting as to the scope and utility of the compounds claimed.

Corticosteroids, also referred to as glucocorticoids, are steroid hormones
that play an important physiological role in mammals, including humans. Control (also
10   referred to as modulation) of glucocorticoid activity is important in regulating
physiological processes in a wide range of tissues and organs.

Glucocorticoid concentrations are modulated by the tissue-specific
11β-hydroxysteroid dehydrogenase enzymes. The two enzymes (also referred to as
isozymes) of 11β-HSD (11β-HSD1 and 11β-HSD2) have different cofactor
15   requirements and substrate affinities (See Figure 1). Each has been successfully cloned
in both rat and human tissues. The 11β-hydroxysteroid dehydrogenase type 2 enzyme
(11β-HSD2) is a high affinity enzyme (K_m for glucocorticoid = 10 nM) that generally
uses NAD+ as the preferred cofactor and rapidly dehydrogenates 11β-hydroxy-
glucocorticoids, such as cortisol, to 11-keto glucocorticoids, such as cortisone. The
20   11β-hydroxysteroid dehydrogenase type 1 enzyme (11β-HSD1) is a low affinity enzyme
that generally uses NADP+ as a cofactor rather than NAD+ (Agarwal *et al.*, 1994, J.
Biol. Chem., 269: 25959-25962). In vitro studies have shown that 11β-HSD1 is
capable of acting as both a reductase and a dehydrogenase. However, 11β-HSD1 in
vivo generally acts as a reductase, converting 11-ketoglucocorticoids, such as cortisone,
25   to 11β-hydroxyglucocorticoids such as cortisol.

Figure 1: 11 Beta-hydroxysteroid Dehydrogenase
Redox Equilibrium of Corticosterolds

Cortisone
(11-dehydro Steroid)
Inactive metabolite

reductase

11-Beta-HSD1

NADPH   NADP+

NADH   NAD+

11-Beta-HSD2

oxidase

Cortisol
(Steroid)
Active metabolite

Glucocorticoid action is mediated by the binding of glucocorticoids to receptors, the most important of which are the mineralocorticoid receptors and

5   glucocorticoid receptors. Mineralocorticoid receptors, through their binding with aldosterone, regulate water and salts in the body and help control the salt-water balance. The mineralocorticoid receptors are non-selective, having an approximately equal affinity for cortisol and aldosterone. Mineralocorticoid receptors are often present in tissues where cortisol is not normally present The 11β-HSD2 enzyme is often present

10   in these same tissues where the mineralocorticoid receptors are located. The 11β-HSD2 enzyme converts cortisol to cortisone, which does not effectively bind to the receptor in competition with aldosterone. This prevents cortisol from binding to the mineralocorticoid receptor, where it would interfere with the regulation of water and salt by aldosterone and the mineralocorticoid receptor.

15   For example, patients suffering from Apparent Mineralocorticoid Excess (AME; see S. Ulick et al., J. Clin. Endocrinol. Metab., 49: 757-763, 1979), a congenital syndrome in which the patient has severe hypertension, have cortisol in the mineralocorticoid receptor target tissues due to reduced activity of the 11β-HSD2 enzyme. Mutations of the gene encoding 11β-HSD2 have been identified in several

20   patients. The cortisol binds to the mineralocorticoid receptor as effectively as aldosterone, causing severe hypertension. The symptoms of AME can also be induced by administration of glycyrrhetinic acid, which is a component of licorice root and which inhibits the 11β-HSD2 enzyme. The glycyrrhetinic acid apparently prevents

conversion of cortisol to cortisone, so that the amount of cortisol available for binding to the mineralocorticoid receptor increases, resulting in hypertension.

The activity of 11β-HSD2 is also high in the placenta. This may protect the fetus from elevated levels of circulating cortisol, which may be detrimental to the health of a developing fetus.

Utilities:

The present invention also relates to the use of a compound of structural formula I or Ia

(I)

wherein:

R^1 is adamantyl, unsubstituted or substituted with one to five substituents independently selected from halogen, OCH_3, OCF_3, CH_3, CF_3, and phenyl, wherein said phenyl is unsubstituted or substituted with one to three halogens;

W is selected from the group consisting of NR^a and a single bond;
X is selected from the group consisting of CH_2 and a single bond;
Z is selected from the group consisting of S and a single bond;

R^a is selected from the group consisting of hydrogen and C_{1-6} alkyl, wherein alkyl is unsubstituted or substituted with one to five fluorines;

R^2 is selected from the group consisting of
    hydrogen,
    C_{1-10} alkyl, unsubstituted or substituted with one to six substituents independently selected from zero to five halogens and zero or one group selected from hydroxy and C_{1-3} alkoxy, said alkoxy group being unsubstituted or substituted with one to three halogens,

C$_{2-10}$ alkenyl, unsubstituted or substituted with one to six substituents

independently selected from zero to five halogens and zero or one group selected from

hydroxy and C$_{1-3}$ alkoxy, said alkoxy group being unsubstituted or substituted with

one to three halogens,

5   CH$_2$CO$_2$H,

CH$_2$CO$_2$C$_{1-6}$ alkyl,

CH$_2$CONHR^a,

(CH$_2$)$_{0-2}$C$_{3-9}$ cycloalkyl,

(CH$_2$)$_{0-2}$C$_{5-12}$ bicycloalkyl,

10   (CH$_2$)$_{0-2}$adamantyl, and

(CH$_2$)$_{0-2}$R;

wherein said C$_{3-9}$ cycloalkyl and C$_{5-12}$ bicycloalkyl optionally have one to two

double bonds, and said C$_{3-9}$ cycloalkyl, C$_{5-12}$ bicycloalkyl, and adamantyl are

unsubstituted or substituted with one to six substituents independently selected from

15   (a) zero to five halogens, CH$_3$, CF$_3$, OCH$_3$, and OCF$_3$, and (b) zero or one phenyl,

said phenyl being unsubstituted or substituted with one to four groups independently

selected from halogen, OCH$_3$, OCF$_3$, CH$_3$, and CF$_3$;

R^3 is selected from the group consisting of

20   hydrogen,

C$_{1-10}$ alkyl, unsubstituted or substituted with one to six substituents

independently selected from zero to five halogens and zero or one group selected from

hydroxy and C$_{1-3}$ alkoxy, said alkoxy group being unsubstituted or substituted with

one to three halogens,

25   C$_{2-10}$ alkenyl, unsubstituted or substituted with one to six substituents

independently selected from zero to five halogens and zero or one group selected from

hydroxy and C$_{1-3}$ alkoxy, said alkoxy group being unsubstituted or substituted with

one to three halogens,

YC$_{3-9}$ cycloalkyl,

30   YC$_{5-12}$ bicycloalkyl,

Yadamantyl, and

YR;

wherein said C$_{3-9}$ cycloalkyl and C$_{5-12}$ bicycloalkyl optionally have one to two

double bonds, and said C$_{3-9}$ cycloalkyl, C$_{5-12}$ bicycloalkyl, and adamantyl are

-21-

unsubstituted or substituted with one to six substituents independently selected from
(a) zero to five halogens, CH3, CF3, OCH3, and OCF3, and (b) zero or one phenyl,
said phenyl being unsubstituted or substituted with one to four groups independently
selected from halogen, OCH3, OCF3, CH3, and CF3;

5

R is selected from the group consisting of benzodioxolane, furan, tetrahydrofuran,
thiophene, tetrahydrothiophene, dihydropyran, tetrahydropyran, pyridine, piperidine,
benzofuran, dihydrobenzofuran, benzothiophene, dihydrobenzothiophene, indole,
dihydroindole, indene, indane, 1,3-dioxolane, 1,3-dioxane, phenyl, and naphthyl;

10   wherein R is unsubstituted or substituted with one to four groups independently
selected from halogen, C_{1-4} alkylthio, C_{1-4} alkylsulfinyl, C_{1-4} alkylsulfonyl, C_{2-4}
alkenylsulfonyl, CN, OH, OCH3, OCF3, and C_{1-4} alkyl, said C_{1-4} alkyl being
unsubstituted or substituted with one to five halogens or one substituent selected from
OH and C_{1-3} alkoxy; and

15

Y is selected from $(CH_2)_{0-2}$ and (-HC=CH-);

or alternatively R^2 and R^3 taken together form a bridging group R^4, providing a
compound of structural formula Ia:

Ia

20

wherein R^4 is
        a C_{2-8} alkylene group, optionally containing one heteroatom selected from O
and NR^b between two adjacent carbon atoms of said C_{2-8} alkylene group, optionally
25   containing one to two carbon-carbon double bonds when R^4 is a C_{3-8} alkylene group,
and optionally also comprising a carbon-carbon single bond connecting two non-
adjacent carbon atoms of said C_{2-8} alkylene group, or
        a C_{4-8} cycloalkyl group;
wherein R^b is selected from the group consisting of hydrogen and C_{1-6} alkyl,
30   unsubstituted or substituted with one to six substituents independently selected from

zero to five fluorines and zero or one phenyl, said phenyl being unsubstituted or substituted with one to three substituents independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3;

wherein R^4 is unsubstituted or substituted with one to five R^c substituents, wherein

5   each R^c is independently selected from halogen, OH, OCH_3, OCF_3, C_{1-6} alkyl, C_{2-6} alkenyl, phenyl, biphenyl, C_{3-8} cycloalkyl, C_{1-6} alkyloxycarbonyl, an epoxide group bridging 2 adjacent carbons, and 1,3-dioxolanyl geminally disubstituted onto one carbon of R^4, wherein each C_{1-6} alkyl and C_{2-6} alkenyl is unsubstituted or substituted with one to five substituents independently selected from zero to three

10   halogens and zero to two groups selected from phenyl, C_{1-6} alkyloxycarbonyl, 1,3-dioxolanyl geminally disubstituted onto one carbon, and CN, and wherein each phenyl, biphenyl, and C_{3-8} cycloalkyl, either as R^c or as a substituent on R^c, is unsubstituted or substituted with one to three groups independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3;

15

wherein R^4 optionally has a fused phenyl ring, a benzodioxinyl ring, or a dihydrobenzodioxinyl ring, said phenyl ring, benzodioxinyl ring, and dihydrobenzodioxinyl ring being unsubstituted or substituted with one to three substituents independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3; and

20

wherein R^4, including said optional fused phenyl ring, benzodioxinyl ring, or dihydrobenzodioxinyl ring and including all substituents on R^4 and said fused phenyl ring, benzodioxinyl ring, or dihydrobenzodioxinyl ring, has no more than 20 carbon atoms;

25

for the inhibition of the reductase activity of 11β-hydroxysteroid dehydrogenase, which is responsible for the conversion of cortisone to cortisol. Excess cortisol is associated with numerous disorders, including NIDDM, obesity, dyslipidemia, insulin resistance, and hypertension. The present invention relates to the use of an 11β-HSD1

30   inhibitor for the treatment, control, amelioration, and/or delay of onset of diseases and conditions that are mediated by excess or uncontrolled amounts of cortisol and/or other corticosteroids in a patient by the administration of a therapeutically effective amount of an 11β-HSD1 inhibitor. Inhibition of the 11β-HSD1 enzyme limits the conversion of cortisone, which is normally inert, to cortisol, which can cause or

contribute to the symptoms of these diseases and conditions if it is present in excessive amounts.

NIDDM, Hypertension. In a second aspect, the compounds of this invention are selective for inhibition of 11β-HSD1 in comparison with 11β-HSD2.

5   Inhibition of 11β-HSD2 can cause serious side effects, such as hypertension. It was previously demonstrated that 11β-HSD1 inhibitors can ameliorate some of the symptoms of NIDDM, such as insulin resistance (B. R. Walker et al., 1995, J. Clin. Endocrinol. Metab., 80: 3155-3159). However, these studies were carried out using glycyrrhetinic acid and carbenoxolone, which are inhibitors of both 11β-HSD1 and

10   11β-HSD2. Glycyrrhetinic acid and carbenoxolone are believed to cause hypertension through the inhibition of 11β-HSD2.

Cortisol is an important and well recognized anti-inflammatory agent. However, cortisol also has detrimental effects if present in large amounts. For example, cortisol acts as an antagonist to the action of insulin in the liver, so that

15   insulin sensitivity is reduced in the liver, resulting in increased gluconeogenesis and elevated levels of glucose in the liver. Therefore, patients who already have impaired glucose tolerance have a greater probability of developing type 2 diabetes in the presence of abnormally high levels of cortisol.

High levels of cortisol in tissues where the mineralocorticoid receptor

20   is present can lead to hypertension, as discussed in the previous section. The 11β-HSD2 enzyme effects the oxidation of cortisol to cortisone. The 11β-HSD1 enzyme acts as a reductase, converting cortisone to cortisol. It has been hypothesized that inhibition of 11β-HSD1 activity will shift the ratio of cortisol and cortisone in specific tissues toward a higher amount of cortisone, which is generally inactive, and a

25   reduced amount of cortisol, which is active and is often the cause of the symptoms. To the extent that elevated cortisol levels can lead to symptoms of Type 2 diabetes, inhibition of the activity of the 11β-HSD1 isozyme should modulate and control the symptoms of type II diabetes. Administration of a therapeutically effective amount of an 11β-HSD1 inhibitor therefore should be effective in treating, controlling, and

30   ameliorating the symptoms NIDDM, and administration of a therapeutically effective amount of an 11β-HSD1 inhibitor on a regular basis may actually delay or prevent the onset of Type II diabetes in a mammalian patient in need thereof, and particularly in a human patient.

Cushing's Syndrome. The effect of elevated levels of cortisol is also

35   observed in patients who have Cushing's syndrome, which is a metabolic disease

characterized by high levels of cortisol in the blood stream. Patients with Cushing's syndrome often develop Type 2 diabetes.

      <u>Obesity, Metabolic Syndrome, Dyslipidemia.</u> Excessive levels of cortisol have been associated with obesity, perhaps due to increased hepatic

5   gluconeogenesis. Abdominal obesity is closely associated with glucose intolerance, hyprinsulinemia, hypertriglyceridemia, and other factors of Syndrome X, such as high blood pressure, elevated VLDL, and reduced HDL. Montague et al., Diabetes, 2000, 49: 883-888. Thus, the administration of an effective amount of an 11β-HSD1 inhibitor may be useful in the treatment or control of obesity by controlling cortisol,

10   independent of its effectiveness in treating NIDDM. Long-term treatment with an 11β-HSD1 inhibitor may also be useful in delaying the onset of obesity, or perhaps preventing it entirely, especially if the patient uses an 11β-HSD1 inhibitor in combination with controlled diet and exercise.

      By reducing insulin resistance and maintaining serum glucose at

15   normal concentrations, compounds of this invention may also have utility in the treatment and prevention of the numerous conditions that often accompany Type II diabetes and insulin resistance, including the metabolic syndrome ("Syndrome X"), obesity, reactive hypoglycemia, and diabetic dyslipidemia.

<u>Other Utilities:</u>

20   The following diseases, disorders and conditions are related to Type 2 diabetes, and some or all of these may be treated, controlled, or in some cases prevented or at least have their onset delayed, by treatment with the compounds of this invention: (1) hyperglycemia, (2) low glucose tolerance, (3) insulin resistance, (4) obesity, (5) lipid disorders, (6) dyslipidemia, (7) hyperlipidemia, (8)

25   hypertriglyceridemia, (9) hypercholesterolemia, (10) low HDL levels, (11) high LDL levels, (12) atherosclerosis and its sequelae, (13) vascular restenosis, (14) pancreatitis, (15) abdominal obesity, (16) neurodegenerative disease, (17) retinopathy, (18) nephropathy, (19) neuropathy, (20) Syndrome X, and other disorders where insulin resistance is a component.

30   <u>Cognition and Dementia.</u> There are also data indicating that excessive levels of cortisol in the brain may result in neuronal loss and neuronal dysfunction through the potentiation of neurotoxins. There have been suggestions in the literature that the cognitive impairment that sometimes is associated with aging may also be associated with excess levels of cortisol in the brain. See J. R. Seckl and B.

35   R.Walker, Endocrinology, 2001, 142: 1371-1376, and references cited therein.

Therefore, administration of an effective amount of an 11β-HSD1 inhibitor may result in the reduction, amelioration, control or prevention of cognitive impairment associated with aging and of neuronal dysfunction

Atherosclerosis. As described above, inhibition of 11β-HSD1 activity
5   and a reduction in the amount of cortisol can also be beneficial in treating or controlling hypertension, which otherwise can result from uncontrolled levels of cortisol. Since hypertension and dyslipidemia contribute to the development of atherosclerosis, administration of a therapeutically effective amount of an 11β-HSD1 inhibitor of this invention may be especially beneficial in treating, controlling,
10   delaying the onset of, or preventing atherosclerosis.

Effects on Pancreas. Inhibition of 11β-HSD1 activity in isolated murine pancreatic β-cells improves glucose stimulated insulin secretion (B. Davani et al., J. Biol. Chem., 2000, 275: 34841-34844). Glucocorticoids were previously shown to reduce insulin secretion in vivo. (B. Billaudel et al., Horm. Metab. Res.,
15   1979, 11: 555-560).

Reduction of Intraocular Pressure. Recent data suggests a connection between the levels of glucocorticoid target receptors and the 11β-HSD enzymes and the susceptibility to glaucoma (J. Stokes et al., Invest. Ophthamol., 2000, 41: 1629-1638). Therefore, inhibition of 11β-HSD1 activity may be useful in reducing
20   intraocular pressure in the treatment of glaucoma.

Immunomodulation. In certain disease states, such as tuberculosis, psoriasis, and stress in general, high glucocorticoid activity shifts the immune response to a humoral response, when in fact a cell based response may be more beneficial to theh patient. Inhibition of 11β-HSD1 activity may reduce glucocorticoid
25   levels, such as cirtisol, thereby shifting the immune response to a cell based response. See D. Mason, Immunology Today, 1991, 12: 57-60, and G.A.W. Rook, Baillièr's Clin,Endocrinol. Metab., 1999, 13: 576-581.

Osteoporosis. Glucocorticoids can inhibit bone formation, which can result in a net bone loss. Other data suggest that 11β-HSD1 may have a role in bone
30   resorption. It therefore appears that inhibition of 11β-HSD1 may be beneficial in preventing bone loss due to osteoporosis. See C.H.Kim et al., J. Endocrinol., 1999, 162: 371-379; C.G.Bellows et al., Bone, 1998, 23: 119-125; and M.S.Cooper et al., Bone, 2000, 27: 375-381.

The above utilities are all believed to be achieved by treatment with
35   11β-HSD1 inhibitors. Since concurrent inhibition of 11β-HSD2 may have deleterious

side effects or may actually increase the amount of cortisol in the target tissue where reduction of cortisol is desired, selective inhibition of 11β-HSD1 activity with little or no inhibition of 11β-HSD2 activity is even more desirable. This need has not been recognized to date, and neither natural nor synthetic selective 11β-HSD1 inhibitors

5   have been identified. Furthermore, the use of selective inhibitors of 11β-HSD1 has not been described.

The 11β-HSD1 inhibitors of this invention generally have an inhibition constant IC_{50} of less than 500 nM, and preferably less than 100 nM. The compounds preferably are selective, having an inhibition constant IC_{50} against 11β-HSD2

10   greater than 500 nM, and preferably greater than 1000 nM. Generally, the IC_{50} ratio for 11β-HSD2 to 11β-HSD1 of a compound is at least two or more, and preferably ten or greater. Even more preferred are compounds with an IC_{50} ratio for 11β-HSD2 to 11β-HSD1 of 100 or greater.

15   Combination Therapy:

Compounds of structural formula I may be used in combination with one or more other drugs in the treatment, prevention, suppression or amelioration of diseases or conditions for which compounds of Structural formula I or the other drugs have utility. Typically the combination of the drugs is safer or more effective than

20   either drug alone, or the combination is safer or more effective than would be expected based on the additive properties of the individual drugs. Such other drug(s) may be administered, by a route and in an amount commonly used contemporaneously or sequentially with a compound of structural formula I. When a compound of structural formula I is used contemporaneously with one or more other drugs, a

25   combination product containing such other drug(s) and the compound of structural formula I is preferred. However, combination therapy also includes therapies in which the compound of structural formula I and one or more other drugs are administered on different overlapping schedules. It is contemplated that when used in combination with other active ingredients, the compound of the present invention or

30   the other active ingredient or both may be used effectively in lower doses than when each is used alone. Accordingly, the pharmaceutical compositions of the present invention include those that contain one or more other active ingredients, in addition to a compound of structural formula I.

Examples of other active ingredients that may be administered in combination with a compound of structural formula I, and either administered separately or in the same pharmaceutical composition, include, but are not limited to:

(a) dipeptidyl peptidase IV (DP-IV) inhibitors;

(b) insulin sensitizers including (I) PPARγ agonists such as the glitazones (e.g. troglitazone, pioglitazone, englitazone, MCC-555, rosiglitazone, and the like) and other PPAR ligands, including PPARα/γ dual agonists, such as KRP-297, and PPARα agonists such as gemfibrozil, clofibrate, fenofibrate and bezafibrate, and (ii) biguanides, such as metformin and phenformin;

(c) insulin or insulin mimetics;

(d) sulfonylureas and other insulin secretagogues such as tolbutamide, glipizide, meglitinide and related materials;

(e) α-glucosidase inhibitors (such as acarbose);

(f) glucagon receptor antagonists such as those disclosed in WO 98/04528, WO 99/01423, WO 00/39088 and WO 00/69810;

(g) GLP-1, GLP-1 mimetics, and GLP-1 receptor agonists such as those disclosed in WO00/42026 and WO00/59887;

(h) GIP, GIP mimetics such as those disclosed in WO00/58360, and GIP receptor agonists;

(i) PACAP, PACAP mimetics, and PACAP receptor 3 agonists such as those disclosed in WO 01/23420;

(j) cholesterol lowering agents such as (I) HMG-CoA reductase inhibitors (lovastatin, simvastatin, pravastatin, cerivastatin, fluvastatin, atorvastatin, itavastatin, rosuvastatin, and other statins), (ii) bile-acid sequestrants (cholestyramine, colestipol, and dialkylaminoalkyl derivatives of a cross-linked dextran), (iii) nicotinyl alcohol, nicotinic acid or a salt thereof, (iv) inhibitors of cholesterol absorption, such as, for example, ezetimibe and beta-sitosterol, (v) acyl CoA:cholesterol acyltransferase inhibitors, such as, for example, avasimibe, and (vi) anti-oxidants, such as probucol;

(k) PPARδ agonists, such as those disclosed in WO97/28149;

(l) antiobesity compounds such as fenfluramine, dexfenfluramine, phentermine, sibutramine, orlistat, neuropeptide Y Y5 antagonists, CB1 receptor inverse agonists and antagonists, β3 adrenergic receptor agonists, and melanocortin-receptor agonists, in particular melanocortin-4 receptor agonists;

(m) an ileal bile acid transporter inhibitor;

-28-

(n) agents intended for use in inflammatory conditions other than glucocorticoids, such as aspirin, non-steroidal anti-inflammatory drugs, azulfidine, and cyclooxygenase 2 selective inhibitors, and

(o) protein tyrosine phosphatase-1B (PTP-1B) inhibitors.

5   The above combinations include a compound of structural formula I, or a pharmaceutically acceptable salt or solvate thereof, not only with one or more other active compounds. Non-limiting examples include combinations of compounds of structural formula I with two or more active compounds selected from biguanides, sulfonylureas, HMG-CoA reductase inhibitors, PPAR agonists, PTP-1B inhibitors,

10   DP-IV inhibitors and anti-obesity compounds.

Administration and Dose Ranges:

Any suitable route of administration may be employed for providing a mammal, especially a human, with an effective dose of a compound of the present invention. For example, oral, rectal, topical, parenteral, ocular, pulmonary, nasal, and

15   the like may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, and the like. Preferably compounds of Formula I are administered orally.

The effective dosage of active ingredient employed may vary depending on the particular compound employed, the mode of administration, the

20   condition being treated and the severity of the condition being treated. Such dosage may be ascertained readily by a person skilled in the art.

When treating or preventing diabetes mellitus and/or hyperglycemia or hypertriglyceridemia or other diseases for which compounds of Formula I are indicated, generally satisfactory results are obtained when the compounds of the

25   present invention are administered at a daily dosage of from about 0.1 milligram to about 100 milligram per kilogram of animal body weight, preferably given as a single daily dose or in divided doses two to six times a day, or in sustained release form. For most large mammals, the total daily dosage is from about 1.0 milligrams to about 1000 milligrams, preferably from about 1 milligrams to about 50 milligrams. In the

30   case of a 70 kg adult human, the total daily dose will generally be from about 7 milligrams to about 350 milligrams. This dosage regimen may be adjusted to provide the optimal therapeutic response.

Pharmaceutical Compositions:

Another aspect of the present invention provides pharmaceutical

35   compositions which comprise a compound of Formula I or Ia, or a pharmaceutically

acceptable salt or prodrug thereof as an active ingredient, and a pharmaceutically acceptable carrier. Optionally other therapeutic ingredients may be included in the pharmaceutical compositions as discussed previously. The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic

5   bases or acids, including inorganic bases or acids and organic bases or acids.

The compositions include compositions suitable for oral, rectal, topical, parenteral (including subcutaneous, intramuscular, and intravenous), ocular (ophthalmic), pulmonary (nasal or buccal inhalation), or nasal administration, although the most suitable route in any given case will depend on the nature and

10   severity of the conditions being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well-known in the art of pharmacy.

In practical use, the compounds of Formula I can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to

15   conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral (including intravenous). In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents

20   and the like in the case of oral liquid preparations, such as, for example, suspensions, elixirs and solutions; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations such as, for example, powders, hard and soft capsules and tablets, with the solid oral preparations being preferred over the liquid

25   preparations.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be coated by standard aqueous or nonaqueous techniques. Such compositions and preparations should

30   contain at least 0.1 percent of active compound. The percentage of active compound in these compositions may, of course, be varied and may conveniently be between about 2 percent to about 60 percent of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that an effective dosage will be obtained. The active compounds can also be administered as intranasal

35   formulations, such as, for example, liquid drops or spray.

The tablets, pills, capsules, and the like may also contain a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin. When a dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier such as a fatty oil.

Various other materials may be present to act as coatings or to modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain, in addition to the active ingredient, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and a flavoring such as cherry or orange flavor.

Compounds of formula I or Ia may also be administered parenterally. Solutions or suspensions of these active compounds can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g. glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

ASSAYS: MEASUREMENT OF INHIBITION CONSTANTS

In vitro enzymatic activity was assessed for test compounds via a Scintillation Proximity Assay (SPA). In short, tritiated-cortisone substrate, NADPH cofactor and titrated compound were incubated with 11β-HSD1 enzyme at 37°C to allow conversion to cortisol to progress. Following this incubation, a preparation of protein A coated SPA beads, pre-blended with anti-cortisol monoclonal antibody and a non-specific 11β-HSD inhibitor, was added to each well. The mixture was shaken at 15°C and was then read on a liquid scintillation counter suitable for 96 well plates.

Percent inhibition was calculated relative to a non-inhibited control well and IC_{50} curves were generated. This assay was similarly applied to 11β-HSD2, whereby tritiated cortisol and NAD were used as the substrate and cofactor, respectively. To begin the assay, 40 μL of substrate (25 nM ³H-Cortisone + 1.25 mM NADPH in 50

5   mM HEPES Buffer, pH 7.4) was added to designated wells on a 96-well plate. Solid compound was dissolved in DMSO at 10 mM followed by a subsequent 50-fold dilution in DMSO. The diluted material was then titrated 4 fold, seven times. 1 μL of each titrated compound was then added in duplicate to the substrate. To start the reaction, 10 μL of 11β-HSD1 microsome from CHO transfectants was added to each

10   well at the appropriate concentration to yield approximately 10% conversion of the starting material. For ultimate calculation of percent inhibition, a series of wells were added that represented the assay minimum and maximum: one set that contained substrate without compound or enzyme (background), and another set that contained substrate and enzyme without any compound (maximum signal). The plates were

15   spun briefly at a low speed in a centrifuge to pool the reagents, sealed with an adhesive strip, mixed gently, and incubated at 37°C for 2 h. After incubation, 45 μL of SPA beads, pre-suspended with anti-cortisol monoclonal antibody and non-specific 11β-HSD inhibitor, were added to each well. The plates were resealed and shaken gently for greater than 1.5 h at 15°C. Data were collected on a plate based liquid

20   scintillation counter such as a Topcount. To control for inhibition of anti-cortisol antibody/cortisol binding, substrate spiked with 1.25 nM ³H cortisol was added to designated single wells. 1 μL of 200 μM compound was added to each of these wells, along with 10 μL of buffer instead of enzyme. Any calculated inhibiton was due to compound interfering with the cortisol binding to the antibody on the SPA beads.

25

ASSAYS: MEASUREMENT OF IN VIVO INHIBITION

In general terms, a test compound was dosed orally to a mammal and a prescribed time interval was allowed to elapse, usually between 1 and 24 hours. Tritiated cortisone was injected intavenously, followed several minutes later by blood

30   collection. Steroids were extracted from the separated serum and analyzed by HPLC. The relative levels of ³H-cortisone and its reduction product, ³H-cortisol, were determined for compound and vehicle-dosed control groups. The absolute conversion, as well as percentage of inhibition, was calculated from these values.

More specifically, compounds were prepared for oral dosing by dissolving them in vehicle (5% hydroxypropyl-beta-cyclodextrin v/v H_2O, or equivalent) at the desired concentration to allow dosing at typically 10 milligrams per kilogram. Following an overnight fasting, the solutions were dosed to ICR mice

5   (obtained from Charles River) by oral gavage, 0.5 mL per dose per animal, with three animals per test group.

After the desired time had passed, routinely either 1 or 4 h, 0.2 mL of 3 μM ^{3}H-cortisone in dPBS was injected by tail vein. The animal was caged for two min followed by euthanasia in a CO_2 chamber. Upon expiration, the mouse was

10   removed and blood was collected by cardiac puncture. The blood was set aside in a serum separation tube for no less than 30 min at room temperature to allow for adequate coagulation. After the incubation period, blood was separated into serum by centrifugation at 3000 x g, 4°C, for 10 min.

To analyze the steroids in the serum they were first extracted with

15   organic solvent. A 0.2 mL volume of serum was transferred to a clean microcentrifuge tube. To this a 1.0 mL volume of ethyl acetate was added, followed by vigorous vortexing for 1 min. A quick spin on a microcentrifuge pelleted the aqueous serum proteins and clarified the organic supernatant. 0.85 mL of the upper organic phase was transferred to a fresh microcentrifuge tube and dried. The dried

20   sample was resuspended in 0.250 mL of DMSO containing a high concentration of cortisone and cortisol for analysis by HPLC.

A 0.200 mL sample was injected onto a Metachem Inertsil C-18 chromatography column equilibrated in 30% methanol. A slow linear gradient to 50% methanol separated the target steroids; simultaneous monitoring by UV at 254

25   nm of the cold standards in the resuspension solution acted as an internal standard. The tritium signal was collected by a radiochromatography detector that uploaded data to software for analysis. The percent conversion of ^{3}H-cortisone to ^{3}H-cortisol was calculated as the ratio of AUC for cortisol over the combined AUC for cortisone and cortisol.

30

In Vivo STUDIES OF UTILITY:

Male db/db mice (10-11 week old C57Bl/KFJ, Jackson Labs, Bar Harbor, ME) were housed 5/cage and allowed *ad lib.* access to ground Purina rodent chow and water. The animals, and their food, were weighed every 2 d and were dosed

35   daily by gavage with vehicle (0.5% carboxymethylcellulose) ± test compound. Drug

suspensions were prepared daily. Plasma glucose and triglyceride concentrations were determined from blood obtained by tail bleeds at 3-5 day intervals during the study period. Glucose and triglyceride, determinations were performed on a Boehringer Mannheim Hitachi 911 automatic analyzer (Boehringer Mannheim,

5 Indianapolis, IN) using heparinized plasma diluted 1:6 (v/v) with normal saline. Lean animals were age-matched heterozygous mice maintained in the same manner.

The following examples are provided so that the invention might be more fully understood. These exampes are illustrative only and should not be

10 construed as limiting the invention in any way.

EXAMPLE 1

Scheme 1

15
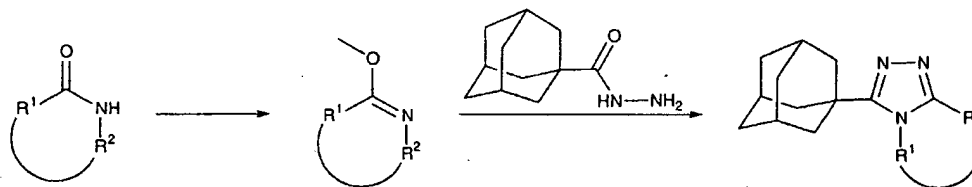
Procedure:

The following compounds were made as part of a one dimensional, single pure compound library on a Myriad Core System. All reaction vessels were

20 dried under a stream of nitrogen at 120 °C for 12 h prior to use. All solvents were dried over sieves for at least 12 h prior to use. All subunits were dissolved in appropriate solvents immediately prior to use.

To each of the reaction vessels was added a methylene chloride solution of the X-component lactams (1.0 mL, 0.10 mmol, 0.1 M in methylene

25 chloride). Next, was added a solution of triethyloxonium tetrafluoroborate (0.120 mL, 0.12 mmol, 1.0 M in methylene chloride). The reactions were aged for 20 h at room temperature. Then a solution of 2,6-di-tert-butyl-4-methylpyridine (0.240 mL, 0.12 mmol, 0.5M in methylene chloride) was added to each vessel. Then the methylene chloride was removed from the reactions via gas agitation. 2 mL of Anhydrous

30 toluene was added to each vessel. Next, a solution of adamantyl hydrazide (1.0 mL,

0.1 mmol, 0.1M in methanol) was added to each vessel. The reactions were then aged for 12 h at 45 °C, followed by heating for 24 h at 120 °C and then cooled to room temperature. Throughout the incubation, the reactions were gas agitated (1 second pulse of nitrogen every hour). Once cooled to room temperature, the crude reaction

5   mixtures were analyzed by LC-MS (Method 1). LC-MS indicated whether or not the desired triazole compounds were formed in the reactions.

All crude reactions were purified by preparative HPLC using mass based detection (Figure 2). The collected fractions were then analyzed for purity by LC-MS; fractions

10   found to be greater than 90% pure were pooled into tared 40 mL EPA vials and lyophilized.

Purification:

15 | Figure 2. FractionLynx HPLC-MS Purification Conditions
|---|
| Column: MetaChem 21 x 100 mm C18-A 5 μm
Flow Rate: 20 mL/min
Pre-inject Equilibration: 0.0 min
Post-inject Hold: 1.0 min
Gradient: 10 to 100% AcCN/water (0.1% TFA) over 6.0 min
Hold: 100 to 100% AcCN/water (0.1% TFA) over 2.0 min
Ramp Back: 100 to 10% AcCN/water (0.1% TFA) over 1.5 min
Total Run time: 10.5 min
Fraction collection triggered by M+1 (ES+) |
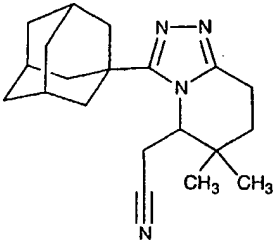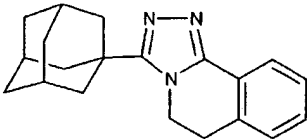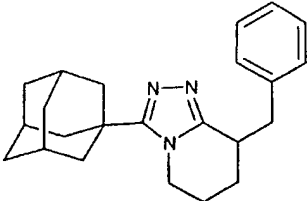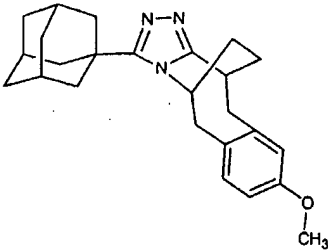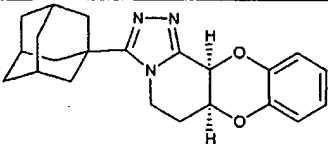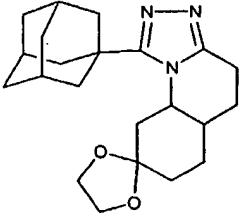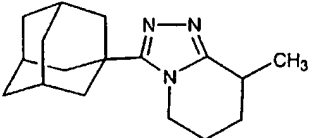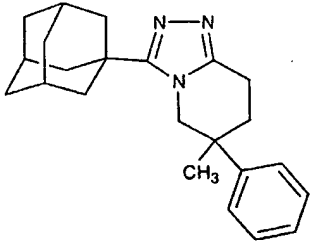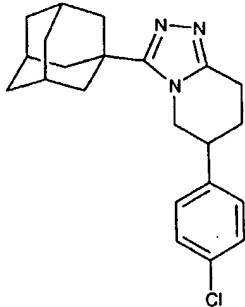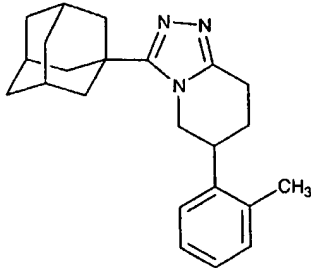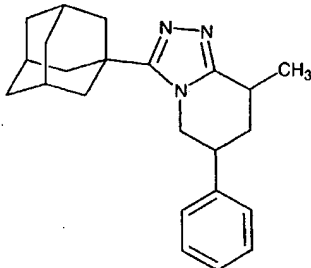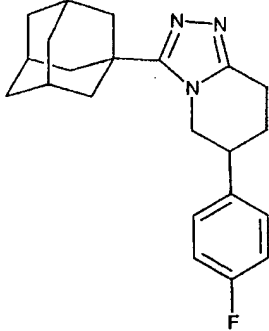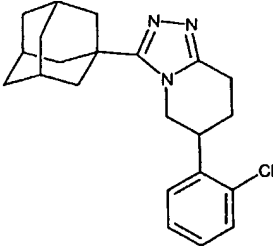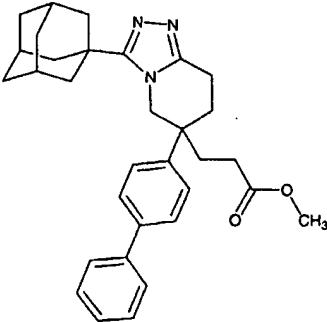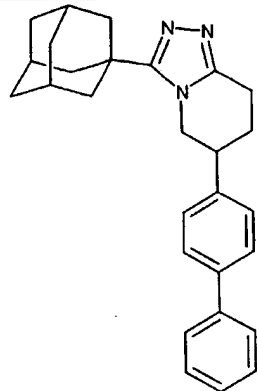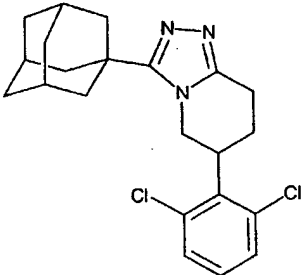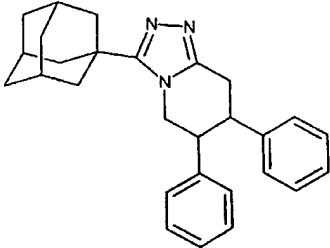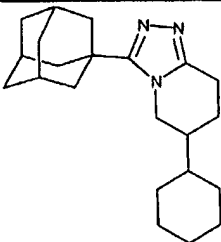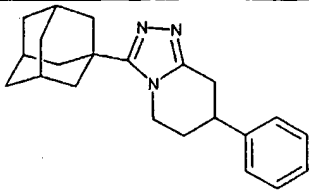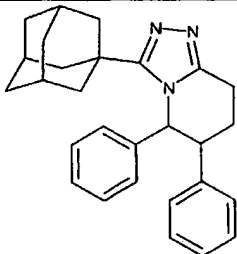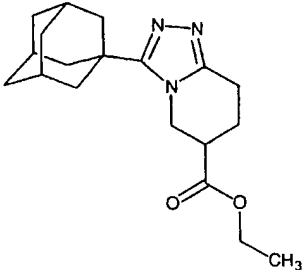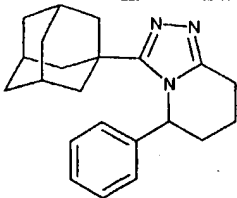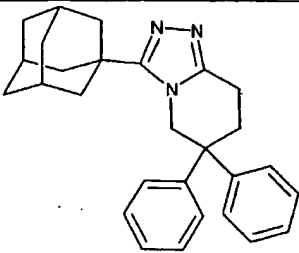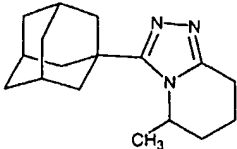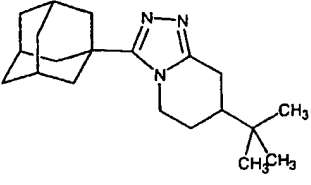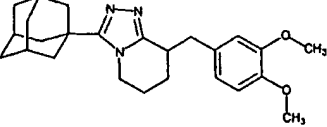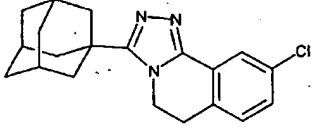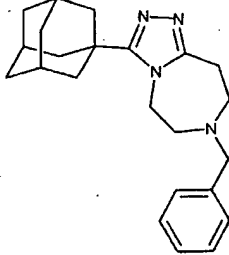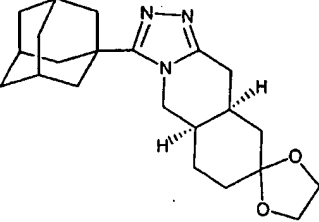
Ex.	Structure	Name	Retention Time (min)	MS ESI (m/z)
1-1		3-(1-adamantyl)-5-(cyanomethyl)-6,6-dimethyl-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt	1.603	325.3
1-2		3-(1-adamantyl)-5,6-dihydro[1,2,4]triazolo[3,4-a]isoquinoline trifluoroacetate salt	1.663	306.1
1-3		3-(1-adamantyl)-8-benzyl-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt	1.807	348.03
1-4		3-(1-adamantyl)-9-methoxy-5,6,11,12-tetrahydro-5,12-ethano[1,2,4]triazolo[4,3-c][3]benzazocine trifluoroacetate salt	1.838	390.5
1-5		(+/-)(6aRS,12aSR)-3-(1-adamantyl)-5,6,6a,12a-tetrahydro[1,4]benzodioxino[2,3-	1.782	363.9
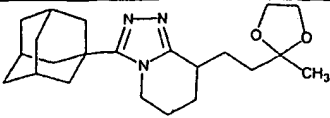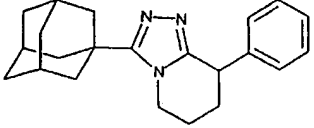
		c][1,2,4]triazolo[4, 3-a]pyridine trifluoroacetate salt		
1-6		1-(1-adamantyl)-5,5a,6,7,9,9a-hexahydro[1,2,4]tri azolo[4,3-a]quinolin-8(4*H*)-one ethylene ketal trifluoroacetate salt	1.682	370.1
1-7		3-(1-adamantyl)-8-methyl-5,6,7,8-tetrahydro[1,2,4]tria zolo[4,3-a]pyridine trifluoroacetate salt	1.497	272.2
1-8		3-(1-adamantyl)-6-methyl-6-phenyl-5,6,7,8-tetrahydro[1,2,4]tria zolo[4,3-a]pyridine trifluoroacetate salt	1.905	348.2
1-9		3-(1-adamantyl)-6-(4-chlorophenyl)-5,6,7,8-tetrahydro[1,2,4]tria zolo[4,3-a]pyridine trifluoroacetate salt	2.013	368.1

1-10		3-(1-adamantyl)-6-(2-methylphenyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt	1.977	348.04
1-11		3-(1-adamantyl)-8-methyl-6-phenyl-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt	1.963	348.3
1-12		3-(1-adamantyl)-6-(4-fluorophenyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt	1.903	352.3
1-13		3-(1-adamantyl)-6-(2-chlorophenyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt	1.985	367.3
1-14		3-(1-adamantyl)-6-(1,1'-biphenyl-4-yl)-6-(3-methoxy-3-oxopropyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt	2.205	496.4

1-15		3-(1-adamantyl)-6-(1,1'-biphenyl-4-yl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyridine trifluoroacetate salt	2.244	410.0
1-16		3-(1-adamantyl)-6-(2,6-dichlorophenyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyridine trifluoroacetate salt	2.044	402.5
1-17		3-(1-adamantyl)-6,7-diphenyl-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyridine trifluoroacetate salt	2.150	410.3
1-18		3-(1-adamantyl)-6-cyclohexyl-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyridine trifluoroacetate salt	2.109	340.4

1-19		3-(1-adamantyl)-7-phenyl-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt	1.812	334.2
1-20		3-(1-adamantyl)-5,6-diphenyl-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt	2.187	410.5
1-21		3-(1-adamantyl)-6-(ethoxycarbonyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt	1.610	330.2
1-22		3-(1-adamantyl)-5-phenyl-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt	1.857	334.0
1-23		3-(1-adamantyl)-6,6-diphenyl-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt	2.123	409.8
1-24		3-(1-adamantyl)-5-methyl-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt	1.531	272.1

1-25		3-(1-adamantyl)-7-*tert*-butyl-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyridine trifluoroacetate salt	1.858	314.2
1-26		3-(1-adamantyl)-8-(3,4-dimethoxybenzyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyridine trifluoroacetate salt	1.778	408.2
1-27		3-(1-adamantyl)-9-chloro-5,6-dihydro[1,2,4]triazolo[3,4-*a*]isoquinoline trifluoroacetate salt	1.793	340.2
1-28		3-(1-adamantyl)-7-benzyl-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*d*][1,4]diazepine bis (trifluoroacetate) salt	1.305	363.5
1-29		(5a*R*,9a*S*)-3-(1-adamantyl)-5,5a,6,7,9a,10-hexahydro[1,2,4]triazolo[4,3-*b*]isoquinolin-8(9*H*)-one ethylene ketal trifluoroacetate salt	1.631	370.4

| 1-30 | | 3-(1-adamantyl)-8-[2-(2-methyl-1,3-dioxolan-2-yl)ethyl]-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt | 2.361 | 394.5 |
| 1-31 | | 3-(1-adamantyl)-8-phenyl-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt | 1.741 | 334.1 |

EXAMPLE 2

Scheme 2
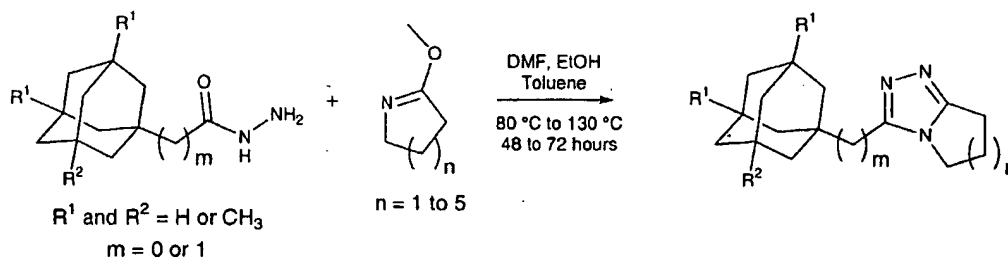
Procedure:

The following compounds were synthesized as part of a 2-D, single, pure compound library using a Myriad Core System. All reaction vessels were dried under a stream of nitrogen at 120 °C for 12 h prior to use. All solvents were dried over sieves for at least 12 h prior to use. All subunits (imino ethers and acyl hydrazides) were dissolved in appropriate solvents immediately prior to use. The following table details the amounts of the subunits and solvents used in the preparation of the library:

-42-

Substance	Amount	Conc.	Mmol	equivalents
Anhydrous Ethanol	2.8 mL	N/A	N/A	N/A
X-axis Iminoether	0.48 mL	0.25 M in Anhydrous Ethanol	0.12	1.2
Y-axis Hydrazide	0.71 mL	0.14 M in 2.5:1 DMF: EtOH	0.10	1.0
Toluene	3 to 4 mL	N/A	N/A	N/A

To 10 mL fritted Myriad reaction vessels under nitrogen was added 2.8 mL of anhydrous ethanol. To each of the reaction vessels was then added an ethanolic

5   solution of the X-component imino ethers (0.48 mL, 0.12 mmol, 0.25 M in ethanol). Next, was added the appropriate Y-component hydrazide (0.71 mL, 0.1 mmol, 0.14 M in 2.5:1 DMF: Ethanol). The reactions were aged for 1 h at room temperature followed by 48 h at 80 °C, after which they were cooled to room temperature. Throughout the incubation, the reactions were gas agitated (1 second pulse of nitrogen

10   every hour). Once cooled to room temperature, the crude reaction mixtures were analyzed by LC-MS (Method 1). LC-MS indicated that the reactions containing 5-methoxy-3,4-dihydro-2H-pyrrole (n=1) had formed adducts with the appropriate hydrazides but failed to dehydrate to the triazole ring; the remaining imino ether based compounds had all formed the desired triazole. The 5-methoxy-3,4-dihydro-2H-

15   pyrrole (n=1) based compounds were returned to their original reaction vessels, diluted to 4 mL total volume with dry toluene, and heated to 130°C for an additional 24 h. Analysis by LC-MS indicated that reactions were complete.

All crude reactions were purified by preparative HPLC using mass based detection (Method 2). The collected fractions were analyzed for purity by LC-

20   MS (Method 3); fractions found to be greater than 90% pure were pooled into tared 40 mL EPA vials and lyophilized.

HPLC Purification Conditions:

Analytical LC Method 1:

5

Column:	MetaChem Polaris C-18A, 30 mm X 4.6 mm, 5.0 μm
Eluent A:	0.1% TFA in Water
Eluent B:	0.1 % TFA in Acetonitrile
Gradient:	5 % B to 95 % B in 3.3 min, ramp back to 5 % B in 0.3

10
	min
Flow:	2.5 mL/min
Column Temperature:	50 °C
Injection amount:	5 μl of undiluted crude reaction mixture.
Detection:	UV at 220 and 254 nm.

15
	MS: API-ES ionization mode, mass scan range (100-600)
	ELSD: Light Scattering Detector

Preparative LC Method 2:

20

Column:	MetaChem Polaris C-18A, 100 mm X 21.2 mm, 10 μm
Eluent A:	0.1% TFA in Water
Eluent B:	0.1 % TFA in Acetonitrile
Pre-inject Equilibration:	1.0 min

25
Post-Inject Hold:	1.0 min
Gradient:	10 % B to 100 % B in 6.0 min, hold at 100 % B for an additional 2.0 min, ramp back from 100% B o 10 % B in 1.5 min
Flow:	20 mL/min

30
Column Temperature:	ambient
Injection amount:	1.5 mL of undiluted crude reaction mixture.
Detection:	MS: API-ES ionization mode, mass scan range (100-600), fraction collection triggered by detection of M+1

Analytical LC Method 3:

Column:	MetaChem Polaris C-18A, 30 mm X 2.0 mm, 3.0 μm
Eluent A:	0.1% TFA in Water
Eluent B:	0.1 % TFA in Acetonitrile
Gradient:	5 % B to 95 % B in 2.0 min, ramp back to 5 % B in 0.1 min
Flow:	1.75 mL/min
Column Temperature:	60 °C
Injection amount:	5 μl of undiluted fraction
Detection:	UV at 220 and 254 nm
	MS: API-ES ionization mode, mass scan range (100-600)
	ELSD: Light Scattering Detector
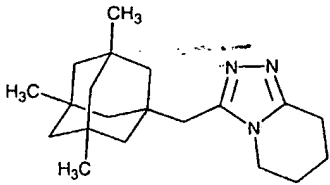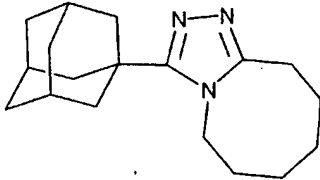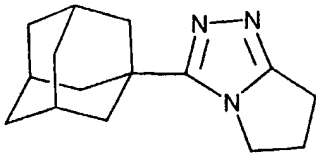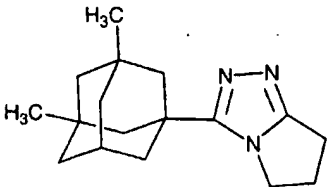
Lyophilization Parameters:
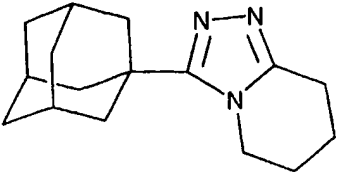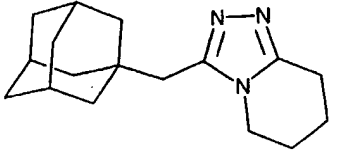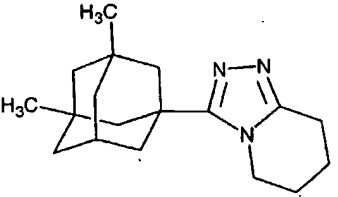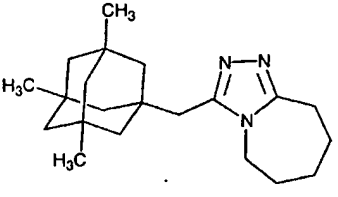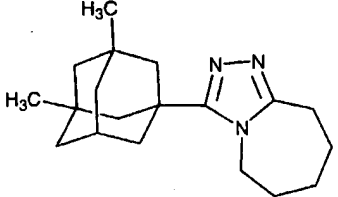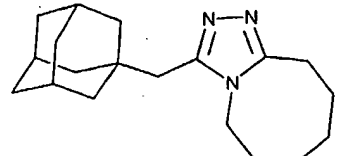
Initial Freeze Setpoint: 1 hour at –70 °C

Drying Phase Condenser Setpoint: -50 °C
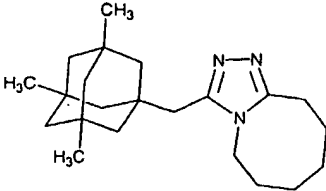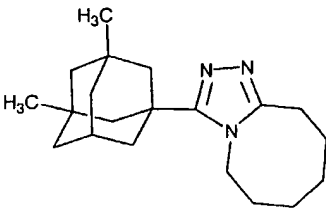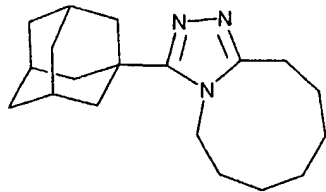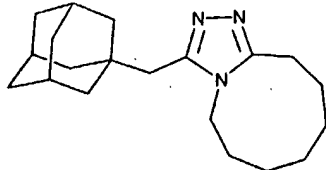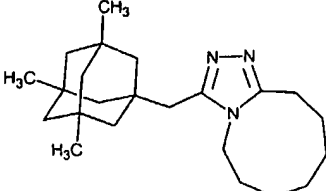
Drying Phase Table:
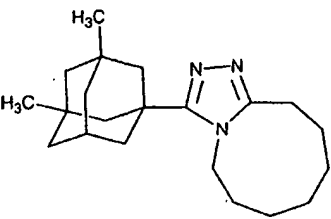
Shelf Temperature (°C)	Duration (min)	Vacuum Setpoint (mTorr)
-60	240	25
-40	240	25
5	480	25
20	1000	25

Table of Compounds:

Ex.	Structure	Name	Retention Time (min)	MS ESI (m/z)
2-1		3-[(3,5,7-trimethyl-1-adamantyl)methyl]-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyridine trifluoroacetate salt	1.982	313.89
2-2		3-(1-adamantyl)-5,6,7,8,9,10-hexahydro[1,2,4]triazolo[4,3-a]azocine trifluoroacetate salt	1.590	285.7
2-3		3-(1-adamantyl)-6,7-dihydro-5H-pyrrolo[2,1-c][1,2,4]triazole trifluoroacetate salt	1.254	243.7
2-4		3-(3,5-dimethyl-1-adamantyl)-6,7-dihydro-5H-pyrrolo[2,1-c][1,2,4]triazole trifluoroacetate salt	1.577	271.92

2-5		3-(1-adamantyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyridine trifluoroacetate salt	1.394	257.54
2-6		3-(1-adamantylmethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyridine trifluoroacetate salt	1.571	271.8
2-7		3-(3,5-dimethyl-1-adamantyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyridine trifluoroacetate salt	1.710	285.5
2-8		3-[(3,5,7-trimethyl-1-adamantyl)methyl]-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepine trifluoroacetate salt	2.048	327.0
2-9		3-(3,5-dimethyl-1-adamantyl)-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepine trifluoroacetate salt	1.773	299.4
2-10		3-(1-adamantylmethyl)-5,6,7,8,9,10-hexahydro[1,2,4]tri	1.739	299.9

		azolo[4,3-*a*]azocine trifluoroacetate salt		
2-11		3-[(3,5,7-trimethyl-1-adamantyl)methyl]-5,6,7,8,9,10-hexahydro[1,2,4]triazolo[4,3-*a*]azocine trifluoroacetate salt	2.126	341.0
2-12		3-(3,5-dimethyl-1-adamantyl)-5,6,7,8,9,10-hexahydro[1,2,4]triazolo[4,3-*a*]azocine trifluoroacetate salt	1.874	313.9
2-13		3-(1-adamantyl)-6,7,8,9,10,11-hexahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azonine trifluoroacetate salt	1.709	299.9
2-14		3-(1-adamantylmethyl)-6,7,8,9,10,11-hexahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azonine trifluoroacetate salt	1.850	313.8
2-15		3-[(3,5,7-trimethyl-1-adamantyl)methyl]-6,7,8,9,10,11-hexahydro-5*H*-[1,2,4]triazolo[4,3-	2.220	355.9

		a]azonine trifluoroacetate salt		
<u>2-16</u>		3-(3,5-dimethyl-1-adamantyl)-6,7,8,9,10,11-hexahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azonine trifluoroacetate salt	1.988	328.1

EXAMPLE 3

Procedure 3A

3-11
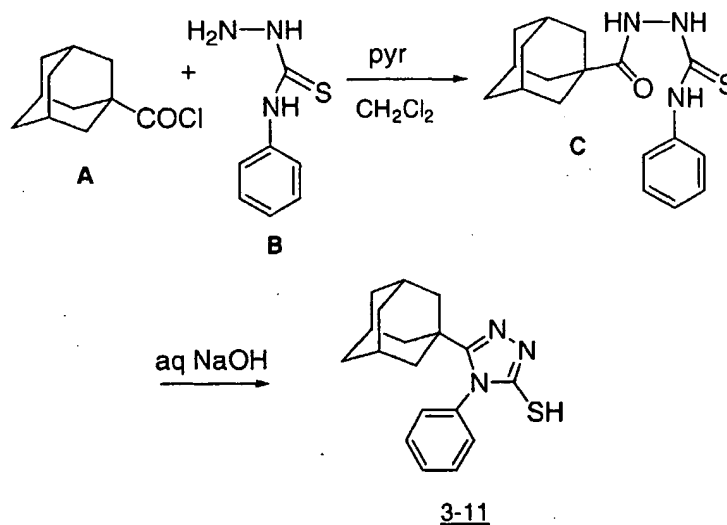
<u>Preparation of 5-(1-adamantyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (3-11)</u>

Pyridine (0.808 mL, 10 mmol) was added dropwise at room temperature to a stirred solution of 1-adamantanecarbonyl choride (**A**) (1 g, 5 mmol) and 4-phenyl-3-thiosemicarbazide (**B**) (0.845 g, 5.05 mmol) in CH_2Cl_2 (10 mL). After stirring for 4 h, the solvent was removed *in vacuo*, and the residue washed with water and dried to give 1-(1-adamantylcarbonyl)-4-phenyl thiosemicarbazide (**C**). MS: 330 (M+1).

A mixture of 1-(1-adamantylcarbonyl)-4-phenylthiosemicarbazide (**C**) (1.48 g) and 2 N NaOH (45 mL) was heated for 1 h under reflux in a N$_2$ atmosphere and filtered. The filtrate was acidified with conc HCl to pH 4. The precipitated solid was filtered, washed with water and dried to give 5-(1-adamantyl)-4-phenyl-4H-1,2,4-
5   triazole-3-thiol (**11**). MS: 312 (M+1).

Compounds **3-10, 3-21, 3-22, 3-25,** and **3-30** were prepared by essentially the same procedure from 1-adamantylcarbonyl chloride and the appropriate 4-substituted-3-thiosemicarbazide.

10   . Procedure 3B
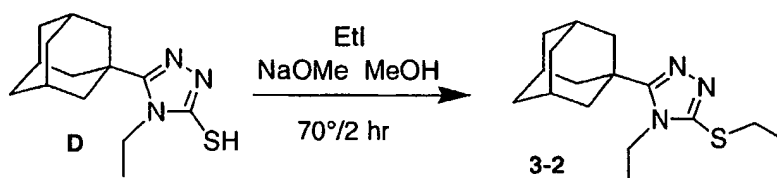
Preparation of 3-(1-adamantyl)-4-ethyl-5-(ethylthio)-4H-1,2,4-triazole (3-2)

5-(1-Adamantyl)-4-ethyl-4H-1,2,4-triazole-3-thiol (**D**, *Arzneim.-*
15   *Forsch.* **1991**, *41*, 1260-1264) (40 mg, 0.15 mmol) and 0.5 M methanolic NaOMe (0.3 mL, 0.15 mmol) in methanol (1 mL) was heated under reflux for 10 min. Ethyl iodide (12 μl, 0.15 mmol) was added, and the mixture was heated under reflux for 2 h. The methanol was removed *in vacuo*, and the residue was partitioned between CH$_2$Cl$_2$ and water. The organic layer was dried (MgSO$_4$) and evaporated *in vacuo*. The residue
20   was purified by chromatography on silica gel with 10% MeOH in CH$_2$Cl$_2$ to give 3-(1-adamantyl)-4-ethyl-5-(ethylthio)-4H-1,2,4-triazole (2), MS: 278 (M+1).

Compounds **3-1** through **3-9, 3-12, 3-13, 3-14, 3-23, 3-24, 3-26** through **3-29, 3-31** through **3-35, 3-40, 3-41; 3-48, 3-49** and **3-50** were prepared by essentially the same procedure from the appropriate 4-substituted 5-(1-adamantyl)-
25   4H-1,2,4-triazole-3-thiol and a bromide or iodide.

Procedure 3C

5

Preparation of 3-(1-adamantyl)-5-(ethylthio)-4H-1,2,4-triazol-4-amine trifluoroacetate salt (3-19)

A mixture of 5-(1-adamantyl)-3-mercapto-4H-1,2,4-triazol-4-amine (**E**, *Chin. Pharm. J.* **1993**, *45*, 101-107) (25 mg, 0.1 mmol), ethyl iodide ((8 μl, 0.1

10   mmol), 0.3 M 1,8-diazabicyclo[5.4.0]non-5-ene (DBU) in DMSO (0.33 mL, 0.1 mmol) in DMSO (0.66 mL) was heated at 65° for 5 h. The reaction mixture was purified directly by reverse phase HPLC on a C-18 silica gel column using an acetonitrile-0.1% trifluoroacetic acid gradient. Fractions containing the product were lyophilized to obtain 3-(1-adamantyl)-5-(ethylthio)-4H-1,2,4-triazol-4-amine

15   trifluoroacetate salt (**19**) MS: 279 (M+1).

Compounds **3-17** to **3-20, 3-39, 3-45, 3-46**, and **3-47** were prepared by essentially the same procedure from the appropriate 4-substituted 5-(1-adamantyl)-4H-1,2,4-triazole-3-thiol and a bromide or iodide. Compound **3-38** was prepared by the same procedure except that twice the amount of DBU was used with 1,3-

20   dibromopropane. The trifluoroacetate salts of compounds **3-15** and **3-16** were converted into the free bases by neutralizing the trifluoroacetic acid with excess aqueous sodium bicarbonate, extraction with CH_2Cl_2, drying ($MgSO_4$), and evaporation *in vacuo*.
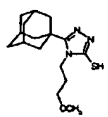
Procedure 3D

Preparation of 4-[3-(1-adamantyl)-5-mercapto-4H-1,2,4-triazol-4-yl]butan-1-ol (3-36)

A mixture of 4-hydroxybutyl isothiocyanate (G, *Synlett.* **1997**, 773-774) (300 mg, 2.3 mmol), 1-adamantanecarbonyl hydrazide (388 mg, 2 mmol) in ethanol (6 mL) was heated under reflux for 1.5 h. After standing overnight at room temperature, the solid was filtered, washed with ethanol and dried to give 1-(1-adamantylcarbonyl)-4-(4-hydroxybutyl) thiosemicarbazide (H). MS: 326 (M+1).

A mixture of 1-(1-adamantylcarbonyl)-4-(4-hydroxybutyl) thiosemicarbazide (H) (471 mg, 1.45 mmol) and 2 N NaOH (12 mL) was heated under reflux in a N$_2$ atmosphere for 1.5 h. The cooled reaction was acidified with conc. HCl to pH 4. The precipitated solid was filtered, washed with water and dried to give 4-[3-(1-adamantyl)-5-mercapto-4H-1,2,4-triazol-4-yl] butan-1-ol (3-36). MS: 308 (M+1).

Compound **3-42** was prepared by essentially the same procedure from 1-adamantylcarbonyl hydrazide and 5-hydroxypentyl isothiocyanate.

Procedure 3E
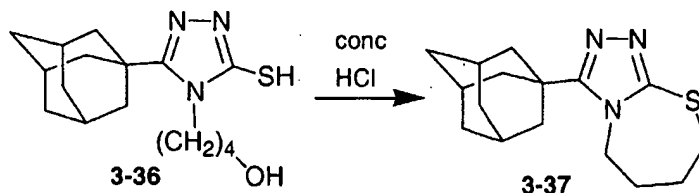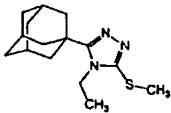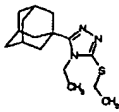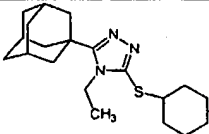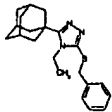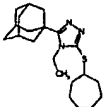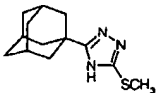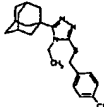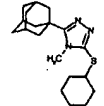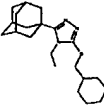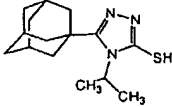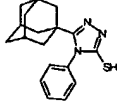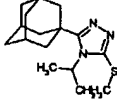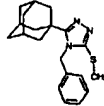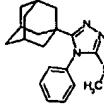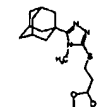
Preparation of 3-(1-adamantyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[3,4-b][1,3]thiazepine (3-37)
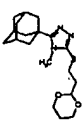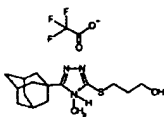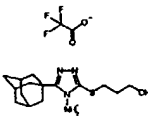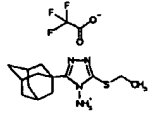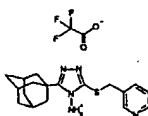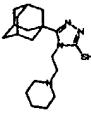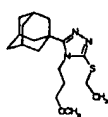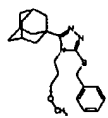
A solution of 4-[3-(1-adamantyl)-5-mercapto-4H-1,2,4-triazol-4-yl]butan-1-ol (3-36) (60 mg) in conc. HCl (6 mL) was heated at 65 °C for 20 h. The
5   cooled solution was added dropwise to 10% aqueous Na₂CO₃ (75 mL). The gum that precipitated was extracted four times with CH₂Cl₂. The combined extracts were dried (MgSO₄) and evaporated *in vacuo*. The residue was purified by reverse phase HPLC on a C-18 silica gel column using an acetonitrile-0.1% trifluoroacetic acid gradient. Fractions containing the product were combined and rendered basic with excess 10%
10   sodium carbonate. After removing most of the acetonitrile *in vacuo*, the basic solution was extracted five times with CH₂Cl₂. The combined extracts were dried (MgSO₄) and evaporated *in vacuo* to give 3-(1-adamantyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[3,4-b][1,3]thiazepine (3-37).
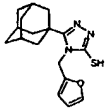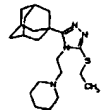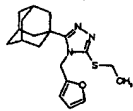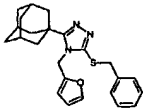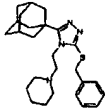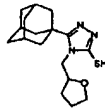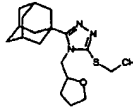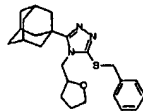
Compound 3-44 was prepared by essentially the same procedure from
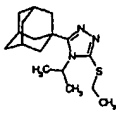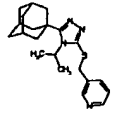15   5-[3-(1-adamantyl)-5-mercapto-4H-1,2,4-triazol-4-yl]pentan-1-ol (3-42).
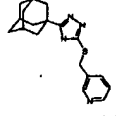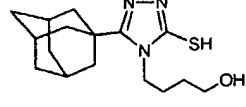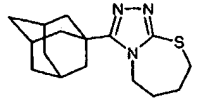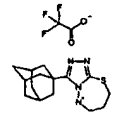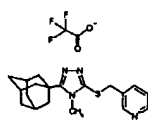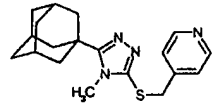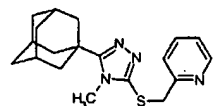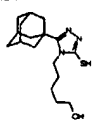
Table of Compounds
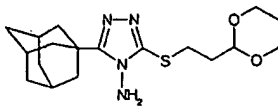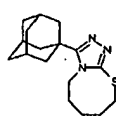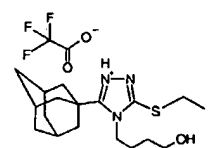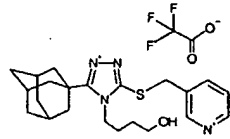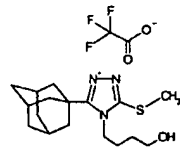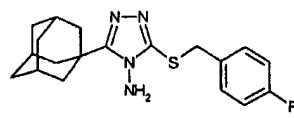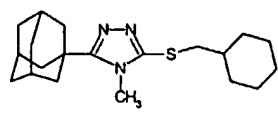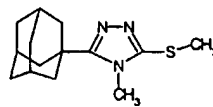
Ex.	Structure	Name	Method	MS ESI (*m/z*)
3-1		3-(1-adamantyl)-4-ethyl-5-(methylthio)-4H-1,2,4-triazole	3B	278
3-2		3-(1-adamantyl)-4-ethyl-5-(ethylthio)-4H-1,2,4-triazole	3B	292
3-3		3-(1-adamantyl)-5-(cyclohexylthio)-4-ethyl-4H-1,2,4-triazole	3B	346
3-4		3-(1-adamantyl)-5-(benzylthio)-4-ethyl-4H-1,2,4-triazole	3B	354

<u>3-5</u>		3-(1-adamantyl)-5-(cycloheptylthio)-4-ethyl-4H-1,2,4-triazole	3B	360
<u>3-6</u>		3-(1-adamantyl)-5-(methylthio)-4H-1,2,4-triazole	3B	250
<u>3-7</u>		3-(1-adamantyl)-5-[(4-chlorobenzyl)thio]-4-ethyl-4H-1,2,4-triazole	3B	388
<u>3-8</u>		3-(1-adamantyl)-5-(cyclohexylthio)-4-methyl-4H-1,2,4-triazole	3B	332
<u>3-9</u>		3-(1-adamantyl)-5-[(cyclohexylmethyl)thio]-4-ethyl-4H-1,2,4-triazole	3B	360
<u>3-10</u>		5-(1-adamantyl)-4-isopropyl-4H-1,2,4-triazole-3-thiol	3A	278
<u>3-11</u>		5-(1-adamantyl)-4-phenyl-4H-1,2,4-triazole-3-thiol	3A	312
<u>3-12</u>		3-(1-adamantyl)-4-isopropyl-5-(methylthio)-4H-1,2,4-triazole	3B	292
<u>3-13</u>		3-(1-adamantyl)-4-benzyl-5-(methylthio)-4H-1,2,4-triazole	3B	340
<u>3-14</u>		3-(1-adamantyl)-4-phenyl-5-(methylthio)-4H-1,2,4-triazole	3B	326
<u>3-15</u>		3-(1-adamantyl)-5-{[2-(1,3-dioxolan-2-yl)ethyl]thio}-4-methyl-4H-1,2,4-triazole	3C	350

3-16		3-(1-adamantyl)-5-{[2-(1,3-dioxan-2-yl)ethyl]thio}-4-methyl-4H-1,2,4-triazole	3C	364
3-17		3-{[5-(1-adamantyl)-4-methyl-4H-1,2,4-triazol-3-yl]thio}propan-1-ol trifluoroacetate salt	3C	308*
3-18		3-{[5-(1-adamantyl)-4-amino-4H-1,2,4-triazol-3-yl]thio}propan-1-ol trifluoroacetate salt	3C	309*
3-19		3-(1-adamantyl)-5-(ethylthio)-4H-1,2,4-triazol-4-amine trifluoroacetate salt	3C	279*
3-20		3-(1-adamantyl)-5-[(pyridin-3-ylmethyl)thio]-4H-1,2,4-triazol-4-amine trifluoroacetate salt	3C	342*
3-21		5-(1-adamantyl)-4-(3-methoxypropyl)-4H-1,2,4-triazole-3-thiol	3A	308
3-22		5-(1-adamantyl)-4-(2-piperidin-1-ylethyl)-4H-1,2,4-triazole-3-thiol	3A	347
3-23		3-(1-adamantyl)-5-(ethylthio)-4-(3-methoxypropyl)-4H-1,2,4-triazole	3B	336
3-24		3-(1-adamantyl)-5-(benzylthio)-4-(3-methoxypropyl)-4H-1,2,4-	3B	398

		triazole		
3-25		5-(1-adamantyl)-4-(2-furylmethyl)-4H-1,2,4-triazole-3-thiol	3A	316
3-26		1-{2-[3-(1-adamantyl)-5-(ethylthio)-4H-1,2,4-triazol-4-yl]ethyl}piperidine	3B	375
3-27		3-(1-adamantyl)-5-(ethylthio)-4-(2-furylmethyl)-4H-1,2,4-triazole	3B	344
3-28		3-(1-adamantyl)-5-(benzylthio)-4-(2-furylmethyl)-4H-1,2,4-triazole	3B	406
3-29		1-{2-[3-(1-adamantyl)-5-(benzylthio)-4H-1,2,4-triazol-4-yl]ethyl}piperidine	3B	437
3-30		5-(1-adamantyl)-4-(tetrahydrofuran-2-ylmethyl)-4H-1,2,4-triazole-3-thiol	3A	332
3-31		3-(1-adamantyl)-5-(ethylthio)-4-(tetrahydrofuran-2-ylmethyl)-4H-1,2,4-triazole	3B	348
3-32		3-(1-adamantyl)-5-(benzylthio)-4-(tetrahydrofuran-2-ylmethyl)-4H-1,2,4-triazole	3B	410

3-33		3-(1-adamantyl)-4-isopropyl-5-(ethylthio)-4H-1,2,4-triazole	3B	306
3-34		3-(1-adamantyl)-4-isopropyl-5-(benzylthio)-4H-1,2,4-triazole	3B	369
3-35		3-({[5-(1-adamantyl)-4H-1,2,4-triazol-3-yl]thio}methyl)pyridine	3B	327
3-36		4-[3-(1-adamantyl)-5-mercapto-4H-1,2,4-triazol-4-yl]butan-1-ol	3D	307
3-37		3-(1-adamantyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[3,4-b][1,3]thiazepine	3E	290
3-38		3-(1-adamantyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[3,4b][1,3,4]thiadiazepine trifluoroacetate salt	3C	291*
3-39		3-({[5-(1-adamantyl)-4-methyl-4H-1,2,4-triazol-3-yl]thio}methyl)pyridine trifluoroacetate salt	3C	341*
3-40		4-({[5-(1-adamantyl)-4-methyl-4H-1,2,4-triazol-3-yl]thio}methyl)pyridine	3B	341
3-41		2-({[5-(1-adamantyl)-4-methyl-4H-1,2,4-triazol-3-yl]thio}methyl)pyridine	3B	341
3-42		5-[3-(1-adamantyl)-5-mercapto-4H-1,2,4-triazol-4-yl]pentan-1-ol	3D	322

3-43		3-(1-adamantyl)-5-{[2-(1,3-dioxan-2-yl)ethyl]thio}-4H-1,2,4-triazol-4-amine	3C	365
3-44		3-(1-adamantyl)-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[3,4-b][1,3]thiazocine	3E	304
3-45		4-[3-(1-adamantyl)-5-(ethylthio)-4H-1,2,4-triazol-4-yl]butan-1-ol trifluoroacetate salt	3C	336*
3-46		4-{3-(1-adamantyl)-5-[(pyridin-3-ylmethyl)thio]-4H-1,2,4-triazol-4-yl}butan-1-ol trifluoroacetate salt	3C	399*
3-47		4-[3-(1-adamantyl)-5-(methylthio)-4H-1,2,4-triazol-4-yl]butan-1-ol trifluoroacetate salt	3C	322*
3-48		3-(1-adamantyl)-5-[(4-fluorobenzyl)thio]-4H-1,2,4-triazol-4-amine	3B	358
3-49		3-(1-adamantyl)-5-[(cyclohexylmethyl)-thio]-4-methyl-4H-1,2,4-triazole	3B	345
3-50		3-(1-adamantyl)-4-methyl-5-(methylthio)-4H-1,2,4-triazole	3B	264
				*free base

EXAMPLE 4

Procedure 4A
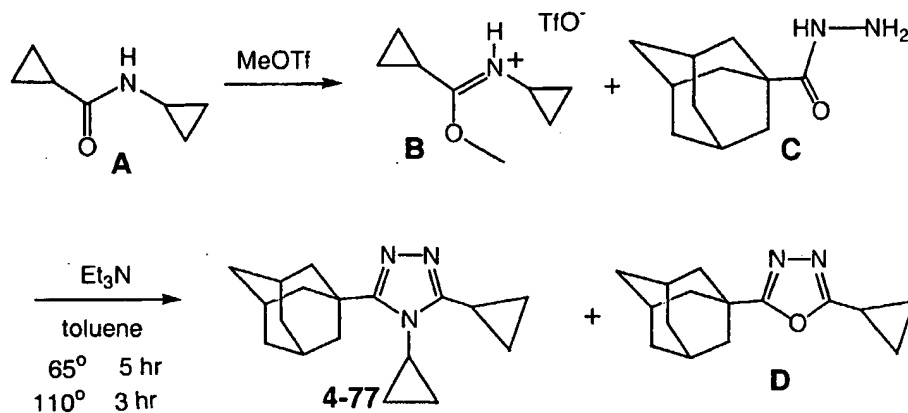
Preparation of 3-(1-adamantyl)-4,5-dicyclopropyl-4H-1,2,4-triazole) (4-77)

A mixture of N-(cyclopropyl)cyclopropanecarboxamide (A) (2.08 g, 16.6 mmol) and methyl trifluoromethanesulfonate (1.88 mL, 16.6 mmol) was warmed at 65° in a nitrogen atmosphere. After a few minutes a clear melt was obtained. After 20 min, the melt was cooled and formation of the imino ether triflate salt (B) confirmed by an NMR spectrum. Toluene (26 mL), triethylamine (3.86 mL, 27.7 mmol) and adamantane-1-carbohydrazide (C) (2.15 g, 11.1 mmol) were added, and the two-phase mixture was stirred at 65° for 5 h. The mixture was heated at 110° for 3 h. The cooled reaction was diluted with ethyl acetate (75 mL), washed with water (75 mL) and saturated brine (30 mL), and dried (MgSO$_4$). The ethyl acetate was evaporated in vacuo to give 2.92 g of a yellow syrup. Flash chromatography on silica gel with ethyl acetate eluted the oxadiazole D. Elution with 7% methanol in chloroform and evaporation in vacuo gave crude 4-77. Recrystallization from isopropyl ether afforded pure 3-(1-adamantyl)-4,5-dicyclopropyl-4H-1,2,4-triazole) (4-77). MS: 284 (M+1).

For less reactive amides a two or three-fold excess of methyl trifluoromethanesulfonate was employed, and the reaction time increased to 1-2 h. The excess methyl trifluoromethanesulfonate was removed in vacuo before addition of the other reagents.

Besides the flash chromatography on silica gel and recrystallization described above, the crude reaction mixtures could be purified by preparative TLC on silica gel or by reverse phase HPLC on a C-18 silica gel column using an acetonitrile-0.1% trifluoroacetic acid gradient or by combinations of these procedures.

5   The amide starting materials that were not available commercially were prepared by EDC/DMAP mediated reaction between the appropriate carboxylic acid and amine in methylene chloride. For N-methyl amides, the appropriate methyl ester or the acid chloride was reacted at room temperature with 40% aqueous methylamine.

10

Procedure 4B

4-55   **4-54**
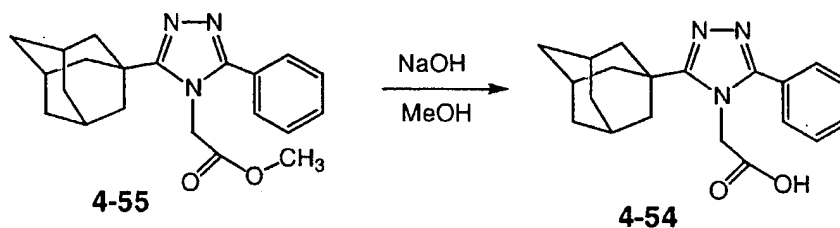
15   Preparation of [3-(1-adamantyl)-5-phenyl-4H-1,2,4-triazol-4-yl]acetic acid (4-54)
          Methyl [3-(1-adamantyl)-5-phenyl-4H-1,2,4-triazol-4-yl]acetate (**4-55**)
(15 mg), 0.5 N NaOH (1 mL) and methanol (0.5 mL) were reacted at room
temperature for 17 h. The methanol was evaporated *in vacuo*. The aqueous residue
was acidified with acetic acid and extracted ten times with chloroform. The extracts
20   were dried (MgSO₄) and evaporated *in vacuo* to give [3-(1-adamantyl)-5-phenyl-4H-
1,2,4-triazol-4-yl]acetic acid (**4-54**). MS: 338 (M+1).

Procedure 4C

4-55   **4-57**
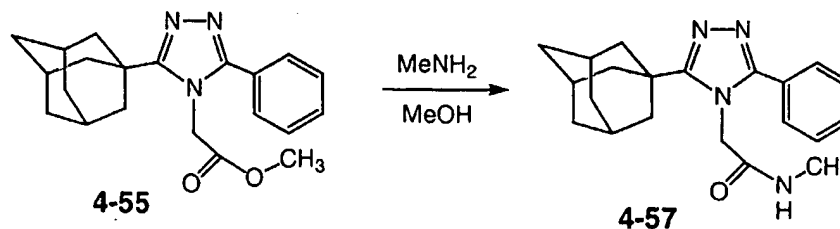
25

Preparation of 2-[3-(1-adamantyl)-5-phenyl-4H-1,2,4-triazol-4-yl]-N-methylacetamide (4-57)

Methyl [3-(1-adamantyl)-5-phenyl-4H-1,2,4-triazol-4-yl]acetate (4-55) (14 mg) and methanol saturated with methylamine at 0° (1 mL) were heated at 65° for 2 h. The mixture was evaporated *in vacuo* to give 2-[3-(1-adamantyl)-5-phenyl-4H-1,2,4-triazol-4-yl]-N-methylacetamide (4-57). MS: 351 (M+1).

Compound 4-56 was prepared by essentially the same procedure from 4-55 and ammonia.

Procedure 4D
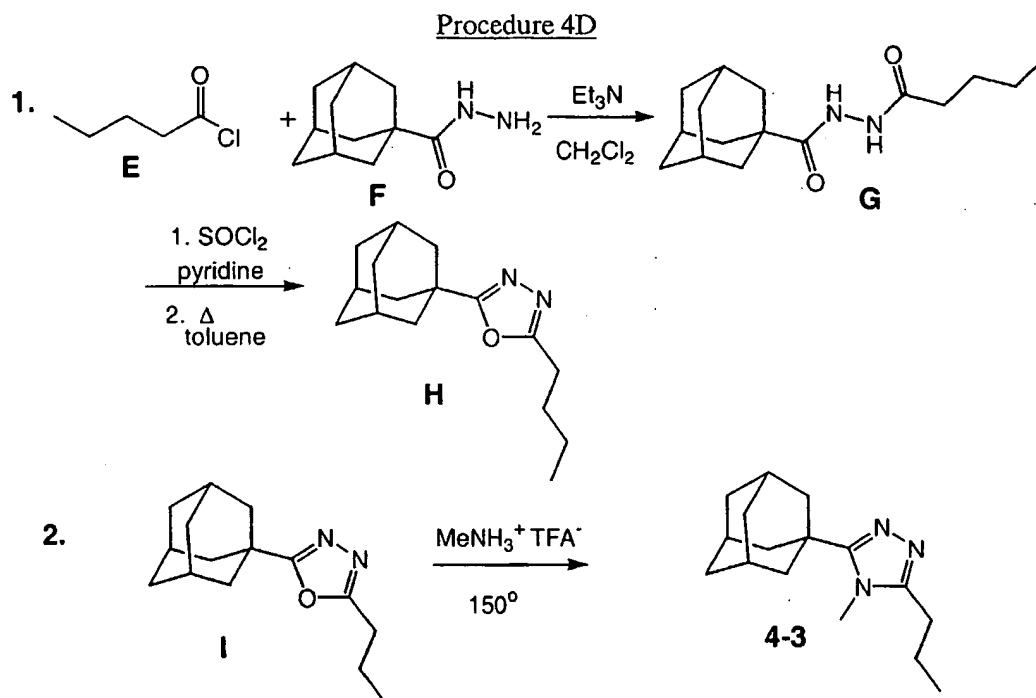
Preparation of 3-(1-adamantyl)-4-methyl-5-propyl-4H-1,2,4-triazole (4-3)

Valeryl chloride (E) (0.981 mL, 8.1 mmol) was added dropwise to a solution of adamantane-1-carbohydrazide (F) (1.5 g, 7.72 mmol) and triethylamine (1.18 mL, 8.49 mmol) in methylene chloride (30 mL) at room temperature, and the mixture stirred at room temperature for 3.5 h. A solution of 10% NaHCO₃ (15 mL) was added and the mixture stirred rapidly for 1.5 h. The mixture was extracted with

methylene chloride (3x) and the combined extracts washed with water, dried (MgSO$_4$) and concentrated *in vacuo* to give *N*'-pentanoyladamantane-1-carbohydrazide (**G**). ^{1}H NMR (CDCl$_3$): δ 0.94 (t, 3H); 1.38 (m, 2H); 1.75 (m, 8H); 1.93 (d, 6H); 2.08 (s, 3H); 2.29 (t, 2H); 8.47 (d, 1H); 8.7 (d,1H).

5   Thionyl chloride (0.71 mL, 9.6 mmol) was added dropwise to a mixture of *N*'-pentanoyladamantane-1-carbohydrazide (**G**) (2.06 g, 7.4 mmol) and pyridine (1.55 mL, 9.2 mmol) at 0°C. After stirring at 0°C for 2.5 h, the mixture was filtered and concentrated *in vacuo*. Toluene (40 mL) was added and the solution refluxed for 3.5 h. The mixture was concentrated *in vacuo* and the residue purified by

10   flash chromatography on silica gel with hexane-ethyl acetate (4:1) to give 2-(1-adamantyl)-5-butyl-1,3,4-oxadiazole (**H**). MS: 261 (M+1).

The oxadiazoles used for the preparation of compounds **4-2, 4-3, 4-4, 4-48, 4-50, 4-58, 4-61, 4-62, 4-63, 4-65, 4-70, 4-71, 4-75, 4-78, 4-88, 4-90, 4-91, 4-98, 4-100,** and **4-109** are prepared essentially by the same procedure from

15   adamantane-1-carbohydrazide and the appropriate acid chloride.

2-(1-Adamantyl)-5-propyl-1,3,4-oxadiazole (**I**) (49 mg, 0.2 mmol) and methylammonium trifluoroacetate (290 mg, 2 mmol, prepared by combining equimolar amounts of methylamine and trifluoroacetic acid in ether followed by concentration *in vacuo*) were stirred together in a sealed vial at 150° for 18 h. The

20   residue was partitioned with methylene chloride and water, the organic layer washed with 10% K$_2$CO$_3$ and brine. The aqueous phase was extracted with methylene chloride (6x), the combined extracts dried (MgSO$_4$) and concentrated *in vacuo*. The residue was purified by reverse phase HPLC on a C-18 silica gel column using an acetonitrile-0.1% trifluoroacetic acid gradient to afford 3-(1-adamantyl)-4-methyl-5-

25   propyl-4H-1,2,4-triazole (**4-3**). MS: 260 (M+1).

Compounds **4-2, 4-3, 4-4, 4-48, 4-50, 4-58, 4-61, 4-62, 4-63, 4-65, 4-70, 4-71, 4-75, 4-78, 4-88, 4-90, 4-91, 4-98, 4-100,** and **4-109** are prepared essentially by the same procedure from an 1,3,4-oxadidazole and the appropriate amine trifluoroacetate salt.

Procedure 4E
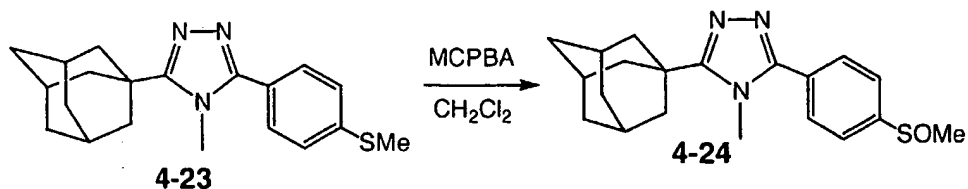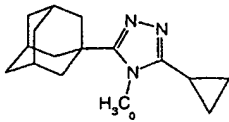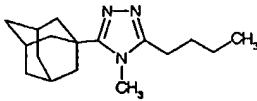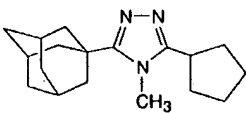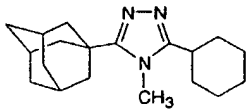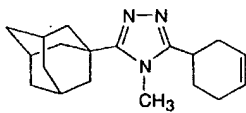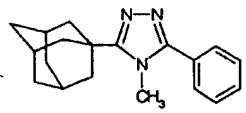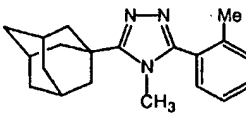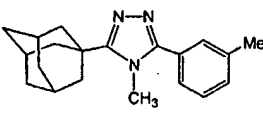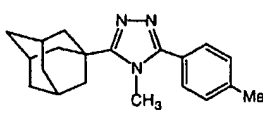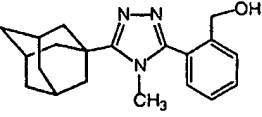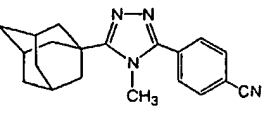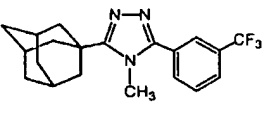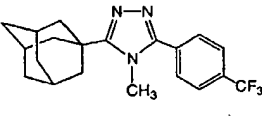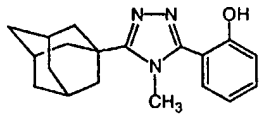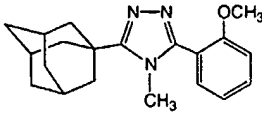
5   Preparation of 3-(1-adamantyl)-4-methyl-5-[4-(methylsulfinyl)phenyl]-4H-1,2,4-
triazole (4-24)
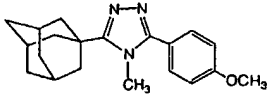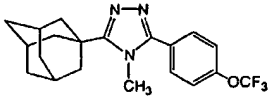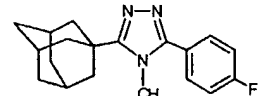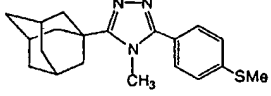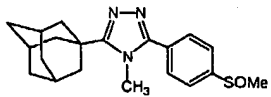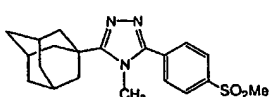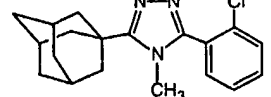
        A mixture of 3-(1-adamantyl)-4-methyl-5-[4-(methylthio)phenyl]-4H-
1,2,4-triazole (4-23) (50 mg, 0.15 mmol) and m-chloroperbenzoic acid (85%,
MCPBA) (45 mg, 0.22 mmol) in methylene chloride (0.75 mL) was stirred at room
10 temperature for 25 min. The mixture was diluted with methylene chloride, washed
with 10% aqueous K_2CO_3, water, and saturated brine and dried ($MgSO_4$). The residue
after evaporation *in vacuo* was purified by reverse-phase chromatography on a C-18
silica gel column with an acetonitrile-0.1% trifluoroacetic acid gradient to give 3-(1-
adamantyl)-4-methyl-5-[4-(methylsulfinyl)phenyl]-4H-1,2,4-triazole (4-24).
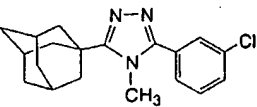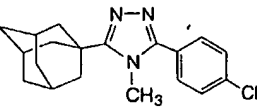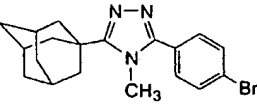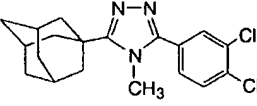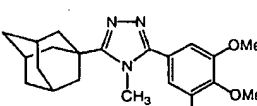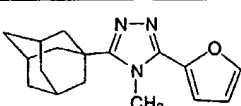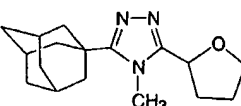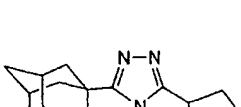
15
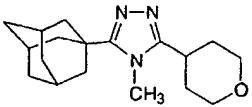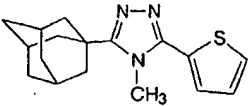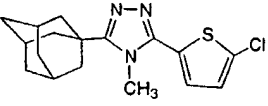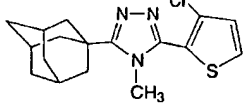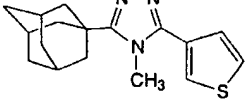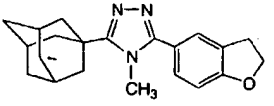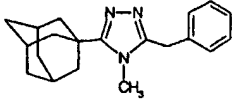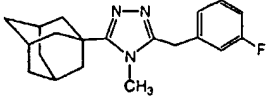
Ex.	Structure	Name	Method	MS ESI (m/z)
4-1		3-(1-adamantyl)-5-(2-methylphenyl)-4H-1,2,4-triazole	4A	294
4-2		3-(1-adamantyl)-4,5-dimethyl-4H-1,2,4-triazole	4D	232
4-3		3-(1-adamantyl)-5-ethyl-4-methyl-4H-1,2,4-triazole	4D	246
4-4		3-(1-adamantyl)-4-methyl-5-propyl-4H-1,2,4-triazole	4D	260
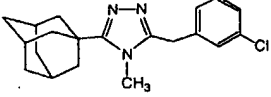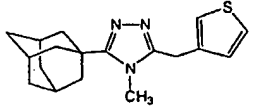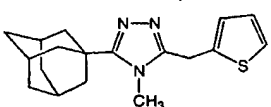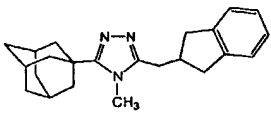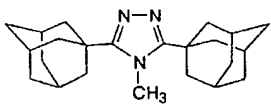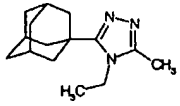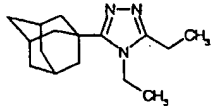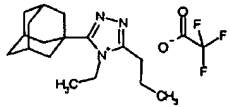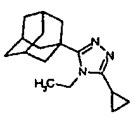
4-5		3-(1-adamantyl)-4-methyl-5-cyclopropyl-4H-1,2,4-triazole	4A	258
4-6		3-(1-adamantyl)-5-butyl-4-methyl-4H-1,2,4-triazole	4A	274
4-7		3-(1-adamantyl)-5-cyclopentyl-4-methyl-4H-1,2,4-triazole	4A	286
4-8		3-(1-adamantyl)-5-cyclohexyl-4-methyl-4H-1,2,4-triazole	4A	300
4-9		3-(1-adamantyl)-5-cyclohex-3-en-1-yl-4-methyl-4H-1,2,4-triazole	4A	298
4-10		3-(1-adamantyl)-5-phenyl-4-methyl-4H-1,2,4-triazole	4A	294
4-11		3-(1-adamantyl)-4-methyl-5-(2-methylphenyl)-4H-1,2,4-triazole	4A	308
4-12		3-(1-adamantyl)-4-methyl-5-(3-methylphenyl)-4H-1,2,4-triazole	4A	308
4-13		3-(1-adamantyl)-4-methyl-5-(4-methylphenyl)-4H-1,2,4-triazole	4A	308

4-14		{2-[5-(1-adamantyl)-4-methyl-4H-1,2,4-triazol-3-yl]phenyl}methanol	4A	324
4-15		4-[5-(1-adamantyl)-4-methyl-4H-1,2,4-triazol-3-yl]benzonitrile	4A	319
4-16		3-(1-adamantyl)-4-methyl-5-[3-(trifluoromethyl)phenyl]-4H-1,2,4-triazole	4A	362
4-17		3-(1-adamantyl)-4-methyl-5-[4-(trifluoromethyl)phenyl]-4H-1,2,4-triazole	4A	362
4-18		2-[5-(1-adamantyl)-4-methyl-4H-1,2,4-triazol-3-yl]phenol	4A	310
4-19		3-(1-adamantyl)-5-(2-methoxyphenyl)-4-methyl-4H-1,2,4-triazole	4A	324
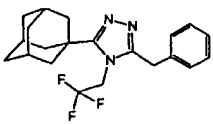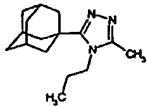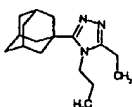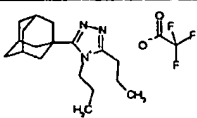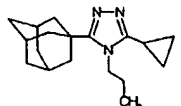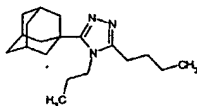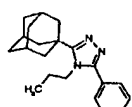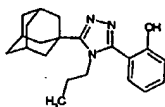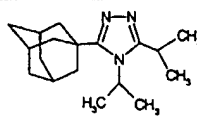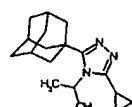
4-20		3-(1-adamantyl)-5-(4-methoxyphenyl)-4-methyl-4H-1,2,4-triazole	4A	324
4-21		3-(1-adamantyl)-4-methyl-5-[4-(trifluoromethoxy)phenyl]-4H-1,2,4-triazole	4A	378
4-22		3-(1-adamantyl)-4-methyl-5-(4-fluorophenyl)-4H-1,2,4-triazole	4A	312
4-23		3-(1-adamantyl)-4-methyl-5-[4-(methylthio)phenyl]-4H-1,2,4-triazole	4A	312
4-24		3-(1-adamantyl)-4-methyl-5-[4-(methylsulfinyl)phenyl]-4H-1,2,4-triazole	4F	356
4-25		3-(1-adamantyl)-4-methyl-5-[4-(methylsulfonyl)phenyl]-4H-1,2,4-triazole	4A	372
4-26		3-(1-adamantyl)-4-methyl-5-(2-chlorophenyl)-4H-1,2,4-triazole	4A	328
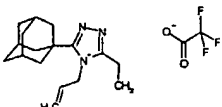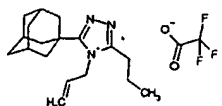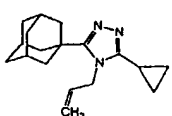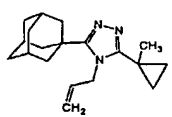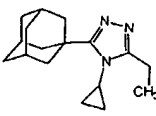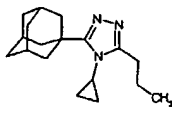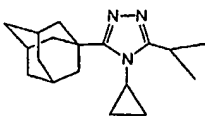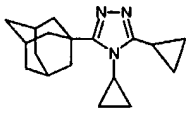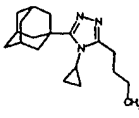
4-27		3-(1-adamantyl)-4-methyl-5-(3-chlorophenyl)-4H-1,2,4-triazole	4A	328
4-28		3-(1-adamantyl)-4-methyl-5-(4-chlorophenyl)-4H-1,2,4-triazole	4A	328
4-29		3-(1-adamantyl)-4-methyl-5-(4-bromophenyl)-4H-1,2,4-triazole	4A	273
4-30		3-(1-adamantyl)-4-methyl-5-(3,4-dichlorophenyl)-4H-1,2,4-triazole	4A	236
4-31		3-(1-adamantyl)-5-(3,4,5-trimethoxyphenyl)-4-methyl-4H-1,2,4-triazole	4A	384
4-32		3-(1-adamantyl)-5-(2-furyl)-4-methyl-4H-1,2,4-triazole	4A	284
4-33		3-(1-adamantyl)-4-methyl-5-tetrahydrofuran-2-yl-4H-1,2,4-triazole	4A	288
4-34		3-(1-adamantyl)-4-methyl-5-tetrahydrofuran-3-yl-4H-1,2,4-triazole	4A	288
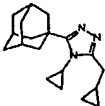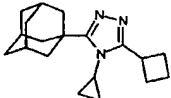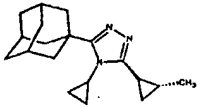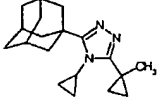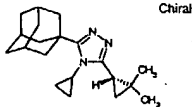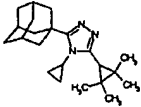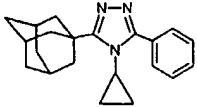
4-35		3-(1-adamantyl)-4-methyl-5-tetrahydro-2H-pyran-4-yl-4H-1,2,4-triazole	4A	302
4-36		3-(1-adamantyl)-5-(2-thienyl)-4-methyl-4H-1,2,4-triazole	4A	300
4-37		3-(1-adamantyl)-5-(5-chlorothien-2-yl)-4-methyl-4H-1,2,4-triazole	4A	334
4-38		3-(1-adamantyl)-5-(3-chlorothien-2-yl)-4-methyl-4H-1,2,4-triazole	4A	334
4-39		3-(1-adamantyl)-5-(3-thienyl)-4-methyl-4H-1,2,4-triazole	4A	300
4-40		3-(1-adamantyl)-5-(2,3-dihydro-1-benzofuran-5-yl)-4-methyl-4H-1,2,4-triazole	4A	336
4-41		3-(1-adamantyl)-5-benzyl-4-methyl-4H-1,2,4-triazole	4A	308
4-42		3-(1-adamantyl)-5-(3-fluorobenzyl)-4-methyl-4H-1,2,4-triazole	4A	326
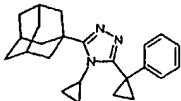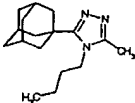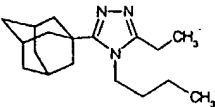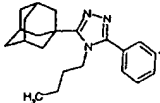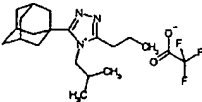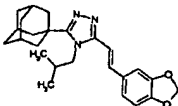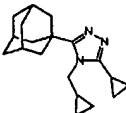
4-43		3-(1-adamantyl)-5-(3-chlorobenzyl)-4-methyl-4H-1,2,4-triazole	4A	342
4-44		3-(1-adamantyl)-4-methyl-5-(thien-3-ylmethyl)-4H-1,2,4-triazole	4A	314
4-45		3-(1-adamantyl)-4-methyl-5-(thien-2-ylmethyl)-4H-1,2,4-triazole	4A	314
4-46		3-(1-adamantyl)-5-(2,3-dihydro-1H-inden-2-ylmethyl)-4-methyl-4H-1,2,4-triazole	4A	348
4-47		3,5-di(1-adamantyl)-4-methyl-4H-1,2,4-triazole	4A	352
4-48		3-(1-adamantyl)-5-methyl-4-ethyl-4H-1,2,4-triazole	4D	246
4-49		3-(1-adamantyl)-4,5-diethyl-4H-1,2,4-triazole	4A	260
4-50		3-(1-adamantyl)-5-propyl-4-ethyl-4H-1,2,4-triazole	4D	274 (free base)
4-51		3-(1-adamantyl)-5-cyclopropyl-4-ethyl-4H-1,2,4-triazole	4A	272
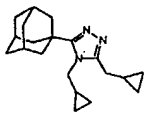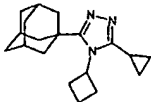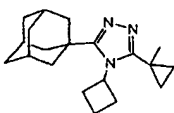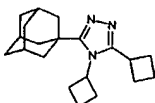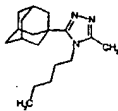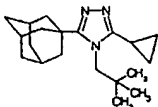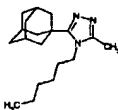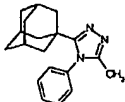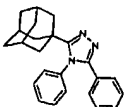
4-52		3-(1-adamantyl)-5-phenyl-4-ethyl-4H-1,2,4-triazole	4A	308
4-53		3-(1-adamantyl)-5-benzyl-4-ethyl-4H-1,2,4-triazole	4A	322
4-54		[3-(1-adamantyl)-5-phenyl-4H-1,2,4-triazol-4-yl]acetic acid	4B	338
4-55		methyl [3-(1-adamantyl)-5-phenyl-4H-1,2,4-triazol-4-yl]acetate	4A	352
4-56		2-[3-(1-adamantyl)-5-phenyl-4H-1,2,4-triazol-4-yl]acetamide	4C	337
4-57		2-[3-(1-adamantyl)-5-phenyl-4H-1,2,4-triazol-4-yl]-N-methylacetamide	4C	351
4-58		3-(1-adamantyl)-5-ethyl-4-(2,2,2-trifluoroethyl)-4H-1,2,4-triazole	4A	314
4-59		3-(1-adamantyl)-5-phenyl-4-(2,2,2-trifluoroethyl)-4H-1,2,4-triazole	4A	362
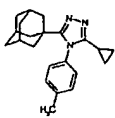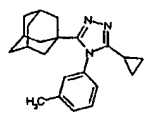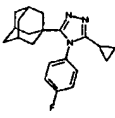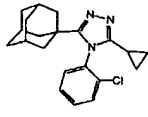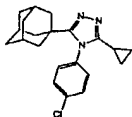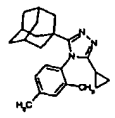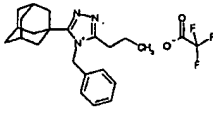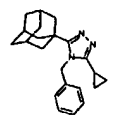
4-60		3-(1-adamantyl)-5-benzyl-4-(2,2,2-trifluoroethyl)-4H-1,2,4-triazole	4A	376
4-61		3-(1-adamantyl)-5-methyl-4-propyl-4H-1,2,4-triazole	4D	260
4-62		3-(1-adamantyl)-5-ethyl-4-propyl-4H-1,2,4-triazole	4D	274
4-63		3-(1-adamantyl)-4,5-dipropyl-4H-1,2,4-triazole	4D	288
4-64		3-(1-adamantyl)-5-cyclopropyl-4-propyl-4H-1,2,4-triazole	4A	286
4-65		3-(1-adamantyl)-5-butyl-4-propyl-4H-1,2,4-triazole	4D	302
4-66		3-(1-adamantyl)-5-phenyl-4-propyl-4H-1,2,4-triazole	4A	322
4-67		2-[5-(1-adamantyl)-4-propyl-4H-1,2,4-triazol-3-yl]phenol	4A	338
4-68		3-(1-adamantyl)-4,5-diisopropyl-4H-1,2,4-triazole	4A	288
4-69		3-(1-adamantyl)-5-cyclopropyl-4-isopropyl-4H-1,2,4-triazole	4A	286
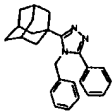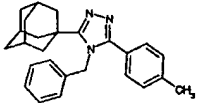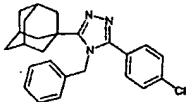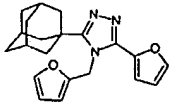
4-70		3-(1-adamantyl)-4-allyl-5-ethyl-4H-1,2,4-triazole	4D	272 (free base)
4-71		3-(1-adamantyl)-4-allyl-5-propyl-4H-1,2,4-triazole	4D	286 (free base)
4-72		3-(1-adamantyl)-4-allyl-5-cyclopropyl-4H-1,2,4-triazole	4A	284
4-73		3-(1-adamantyl)-4-allyl-5-(1-methylcyclopropyl)-4H-1,2,4-triazole	4A	298
4-74		3-(1-adamantyl)-4-cyclopropyl-5-ethyl-4H-1,2,4-triazole	4A	272
4-75		3-(1-adamantyl)-4-cyclopropyl-5-propyl-4H-1,2,4-triazole	4D	286
4-76		3-(1-adamantyl)-4-cyclopropyl-5-isopropyl-4H-1,2,4-triazole	4A	362
4-77		3-(1-adamantyl)-4,5-dicyclopropyl-4H-1,2,4-triazole	4A	284
4-78		3-(1-adamantyl)-4-cyclopropyl-5-butyl-4H-1,2,4-triazole	4A	300

4-79		3-(1-adamantyl)-4-cyclopropyl-5-(cyclopropylmethyl)-4H-1,2,4-triazole	4D	298
4-80		3-(1-adamantyl)-5-cyclobutyl-4-cyclopropyl-4H-1,2,4-triazole	4A	298
4-81		3-(1-adamantyl)-4-cyclopropyl-5-[(1S,2R)-2-methylcyclopropyl]-4H-1,2,4-triazole	4A	298
4-82		3-(1-adamantyl)-4-cyclopropyl-5-(1-methylcyclopropyl)-4H-1,2,4-triazole	4A	298
4-83		3-(1-adamantyl)-4-cyclopropyl-5-[(1S)-2,2-dimethylcyclopropyl]-4H-1,2,4-triazole	4A	312
4-84		3-(1-adamantyl)-4-cyclopropyl-5-(2,2,3,3-tetramethylcyclopropyl)-4H-1,2,4-triazole	4A	340
4-85		3-(1-adamantyl)-4-cyclopropyl-5-phenyl-4H-1,2,4-triazole	4A	320

4-86		3-(1-adamantyl)-4-cyclopropyl-5-benzyll-4H-1,2,4-triazole	4A	334
4-87		3-(1-adamantyl)-4-cyclopropyl-5-(1-phenylcyclopropyl)-4H-1,2,4-triazole	4A	360
4-88		3-(1-adamantyl)-5-methyl-4-butyl-4H-1,2,4-triazole	4D	274
4-89		3-(1-adamantyl)-5-ethyl-4-butyl-4H-1,2,4-triazole	4A	288
4-90		3-(1-adamantyl)-5-phenyl-4-butyl-4H-1,2,4-triazole	4D	336
4-91		3-(1-adamantyl)-4-isobutyl-5-propyl-4H-1,2,4-triazole	4D	302 (free base)
4-92		3-(1-adamantyl)-5-[(E)-2-(1,3-benzodioxol-5-yl)ethenyl]-4-isobutyl-4H-1,2,4-triazole	4A	406
4-93		3-(1-adamantyl)-5-cyclopropyl-4-(cyclopropylmethyl)-4H-1,2,4-triazole	4A	298

4-94		3-(1-adamantyl)-4,5-bis(cyclopropylmethyl)-4H-1,2,4-triazole	4A	312
4-95		3-(1-adamantyl)-4-cyclobutyl-5-cyclopropyl-4H-1,2,4-triazole	4A	245
4-96		3-(1-adamantyl)-4-cyclobutyl-5-(1-methylcyclopropyl)-4H-1,2,4-triazole	4A	312
4-97		3-(1-adamantyl)-4,5-dicyclobutyl-4H-1,2,4-triazole	4A	259
4-98		3-(1-adamantyl)-5-methyl-4-pentyl-4H-1,2,4-triazole	4D	288
4-99		3-(1-adamantyl)-5-cyclopropyl-4-neopentyl-4H-1,2,4-triazole	4A	314
4-100		3-(1-adamantyl)-5-methyl-4-hexyl-4H-1,2,4-triazole	4D	202
4-101		3-(1-adamantyl)-5-methyl-4-phenyl-4H-1,2,4-triazole	4A	294
4-102		3-(1-adamantyl)-4,5-diphenyl-4H-1,2,4-triazole	4A	356

4-103		3-(1-adamantyl)-5-cyclopropyl-4-(4-methylphenyl)-4H-1,2,4-triazole	4A	334
4-104		3-(1-adamantyl)-5-cyclopropyl-4-(3-methylphenyl)-4H-1,2,4-triazole	4A	334
4-105		3-(1-adamantyl)-5-cyclopropyl-4-(4-fluorophenyl)-4H-1,2,4-triazole	4A	338
4-106		3-(1-adamantyl)-5-cyclopropyl-4-(2-chlorophenyl)-4H-1,2,4-triazole	4A	354
4-107		3-(1-adamantyl)-5-cyclopropyl-4-(4-chorophenyl)-4H-1,2,4-triazole	4A	354
4-108		3-(1-adamantyl)-5-cyclopropyl-4-(2.4-dimethylphenyl)-4H-1,2,4-triazole	4A	348
4-109		3-(1-adamantyl)-4-benzyl-5-propyl-4H-1,2,4-triazole	4D	336 (free base)
4-110		3-(1-adamantyl)-4-benzyl-5-cyclopropyl-4H-1,2,4-triazole	4A	334

4-111		3-(1-adamantyl)-4-benzyl-5-phenyl-4H-1,2,4-triazole	4A	370
4-112		3-(1-adamantyl)-4-benzyl-5-(4-methylphenyl)-4H-1,2,4-triazole	4A	384
4-113		3-(1-adamantyl)-4-benzyl-5-(4-chlorophenyl)-4H-1,2,4-triazole	4A	404
4-114		3-(1-adamantyl)-5-(2-furyl)-4-(2-furylmethyl)-4H-1,2,4-triazole	4A	350

EXAMPLE 5-1
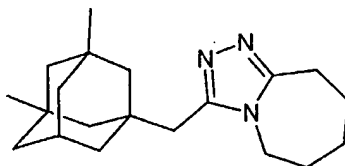
Preparation of 3-[(3,8-dimethyladamantanyl)methyl]-4H,5H,6H,7H,8H-1,2,4-triazolo[4,3-a]perhydroazepine (5-1)

Concentrated sulfuric acid (44 mL) and boron trifluoride etherate (3.53 mL) were added to a flask and cooled to 8 °C. A solution of 1-bromo-3,5-dimethyladamantane (11.02 g) in 1,1-dichloroethylene (35.3 mL) was added dropwise over a 2 hour period. The temperature was kept between 14 and 18 °C and gas evolution was observed. After stirring 1 hour at 10 °C, the reaction was worked up by adding to ice and extracting with diethyl ether. The organic layer was extracted with 1N NaOH (3X), and the combined aqueous solution was acidified with sulfuric acid and re-extracted with ether (3X). The organic layers were combined, dried over

magnesium sulfate, filtered and evaporated to dryness to give crude 3,5-dimethyladamantaneacetic acid (6.23 g).

　　　　3,5-Dimethyladamantaneacetic acid (1.515 g) was dissolved in methylene chloride (50 mL) and stirred at room temperature under nitrogen. Oxalyl

5　chloride (2.38 mL) was added and the reaction was stirred for 2 h whereupon all of the volatiles were removed. The crude acid chloride was dissolved in THF (30 mL) and added to a stirring solution of hydrazine (5 mL), methanol (5 mL), and THF (5 mL). The methanol and THF were removed by evaporation and the remaining liquid was added to aqueous NaOH (1N) and extracted with ethyl acetate (4X). The organic

10　layers were combined, dried over magnesium sulfate, filtered and evaporated to give 2-(3,5-dimethyl-1-adamantyl)acetohydrazide as a clear thick oil (1.60 g).

　　　　The acyl hydrazide (0.85 g), 1-aza-2-methoxy-1-cycoheptene (559 mg) and anhydrous methanol (10 mL) were added to a flask, warmed to 40 °C and stirred for 1 h. The solution was warmed to 50 °C for 1 h then refluxed overnight. After

15　cooling, the methanol was evaporated and the crude product was purified by column chromatography (silica gel, 100% Ethyl acetate → 10% methanol/ ethyl acetate → 10% methanol/CH$_2$Cl$_2$).

EXAMPLE 5-2

20

95 : 5
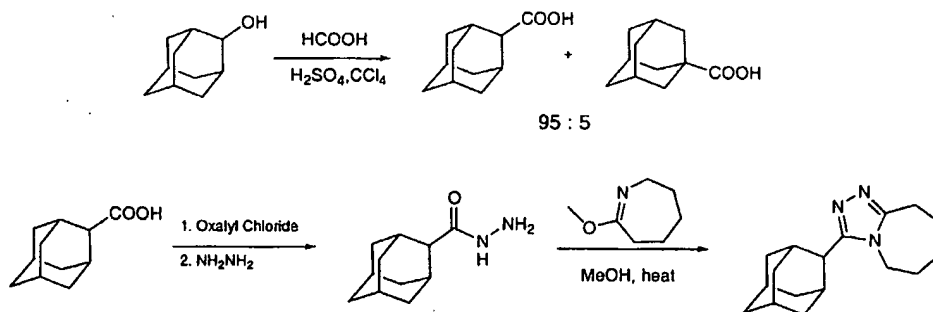
Preparation of 3-adamantan-2-yl-4H,5H,6H,7H,8H-1,2,4-triazolo[4,3-a]perhydroazepine (5-2)

25　　　　Concentrated sulfuric acid (50 mL) and carbon tetrachloride (100 mL) were combined, cooled to 0 °C and vigorously stirred. Adamantan-2-ol (451 mg) was dissolved in 96% formic acid (6 mL) and the solution was added to the sulfuric acid over 1 hour. The reaction continued to stir at 0 °C for 90 min after which it was

added to 300 mL of ice. The layers were separated and the aqueous layer was extracted with 50 mL carbon tetrachloride (2X). The organic layers were combined and extracted with 1N NaOH. The aqueous portion was extracted with methylene chloride (4X) then acidified with 5N HCl. The solution turned white and was cooled

5   on ice. Filtration provided the desired adamantane-2-carboxylic acid (contaminated with about 5% adamantane-1-carboxylic acid) as a white powder.

        The adamantanecarboxylic acid (372 mg) was added to methylene chloride (9 mL) and stirred at room temperature under nitrogen. Oxalyl chloride (2.38 mL) was added and the reaction was stirred for 2 h whereupon all of the volatiles were

10   removed. The crude acid chloride was dissolved in THF (10 mL) and added to a stirring solution of hydrazine (3.3 mL), methanol (6.6 mL), and THF (4.9 mL) at 0°C. The solution was filtered and added to 0.1N NaOH (in a brine solution) and extracted with ethyl acetate (3X). The organic layers were combined, dried over magnesium sulfate, filtered and evaporated to dryness to give adamantane-2-carbohydrazide as a

15   white powder. The crude acyl hydrazide, 1-aza-2-methoxy-1-cycoheptene (325 µL) and one drop of acetic acid were added to anhydrous toluene (35 mL) and stirred overnight. The solution was then refluxed for 3 h. After cooling, the toluene was evaporated and the crude product was purified by column chromatography (silica gel, 100% Ethyl acetate → 10% methanol/ ethyl acetate → 10% methanol/CH$_2$Cl$_2$).

20

EXAMPLE 5-3
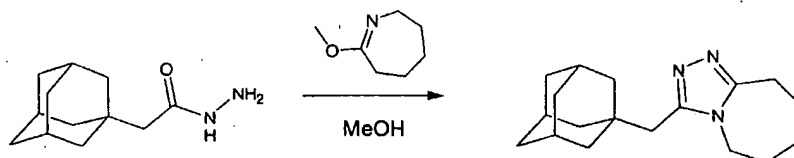
25   Preparation of 3-(adamantanylmethyl)-4H,5H,6H,7H,8H-1,2,4-triazolo[4,3-a]perhydroazepine (5-3)

        2-(1-Adamantyl)acetohydrazide (32.5 mg), 1-aza-2-methoxy-1-cycoheptene (27 µL) and anhydrous methanol (3 mL) were added to a flask, warmed to 50 °C and stirred for 2 h. The solution was then heated to 70 °C for 48 h. After

30   cooling, the methanol was evaporated and the crude product was purified by preparative HPLC to give the trifluoroacetate salt of the title compound as a white powder.

EXAMPLE 5-4
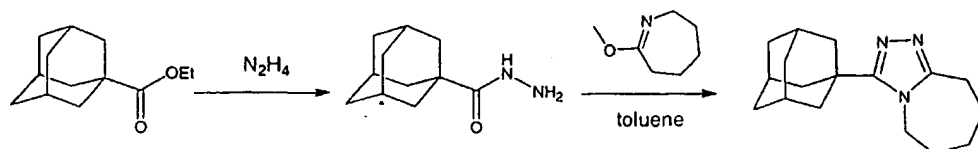
Preparation of 3-adamantanyl-1H,4H,5H,6H,7H,8H-1,2,4-triazolo[4,5-f]azepine (5-4)

A mixture of ethyl 1-adamantanecarboxylate (236.6 g, 1.14 mol), hydrazine hydrate (500 g, about 8.5 mol) and diethylene glycol (2 kg) was refluxed for about 65 h. The solution was allowed to cool to room temperature and aged for 10 days. The resulting suspension was poured into water (6 L) with stirring. The resulting slurry was filtered, and the cake washed with water (900 mL). The cake was re-slurried with water (1 L), filtered and the cake washed with water (1 L) and hexanes (2 L). The solid was air-dried affording 191.7 g of off-white crystalline material.

The hydrazide from above, (90 g, 0.46 mole), 1-aza-2-methoxy-1-cycloheptene (75 mL, 66.5 g, 0.52 mol), acetic acid (1 mL) and toluene (1.35 L) were combined under nitrogen and stirred mechanically. The reaction gradually thickened as a white solid formed. After 20 min, additional toluene (200 mL) was added. The reaction continued to thicken and after another 5 min, additional toluene (300 mL) was added. The reaction thickened and was aged an additional 15 min without agitation. The reaction was diluted with toluene (500 mL) and hexanes (2.5 L), stirred for 5 min then filtered. The cake was washed with 1:1 toluene/hexanes (2 X 350 mL), followed by hexanes (1 L). While the cake was still damp, it was transferred to a flask fitted with a simple distillation head. Toluene (2 L) and acetic acid (1 mL) were added and the mixture heated. Slow distillation of the mixture afforded 500 mL of distillate collected over 1 h, with a distillate temperature of 104°C attained. The solution was cooled and concentrated on a rotary evaporator to a thick slurry (about 200 mL). This was diluted with ether (about 300 mL) and filtered. The cake was washed with 3:1 ether/toluene, ether and dried affording 106.7 g of semi-pure material.

A 24 g sample of comparable semi-pure material obtained from a smaller run was combined with the two crops above and chromatographed (silica 85:15:1 ether/methanol/NH₄OH). The product cuts were concentrated, and the

concentrate flushed with toluene. The residue was diluted with ether (500 mL), cooled to 0°C, aged 30 min and filtered. The cake washed with ether and the product dried affording 122 g of white crystalline material.

500 MHz ^{1}H-NMR (CDCl$_3$): δ 4.17 (br t, 2H), 2.96 (br t, 2H), 2.09 – 2.04 (m, 9H),
5   1.69 – 1.90 (m, 12H).

EXAMPLE 5-5
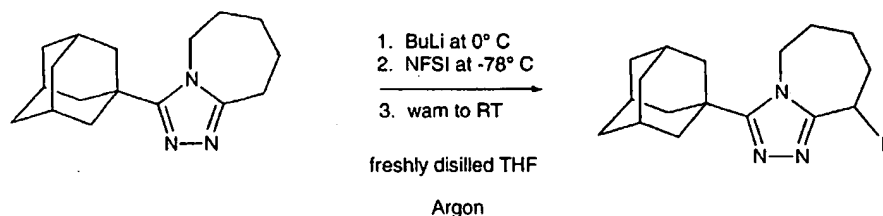
10

Preparation of 3-adamantanyl-8-fluoro-4H,5H,6H,7H,8H-1,2,4-triazolo[4,3-
a]perhydroazepine (5-5)

    3-(1-Adamantyl)-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepine
(105.2 mg) was dissolved in anhydrous THF and cooled to 0 °C while stirring under
15   argon. N-Butyllithium (0.29 mL, 1.6M solution in hexanes) was added and the
solution turned bright yellow and was cooled to –77 °C. N-fluorobenzenesulfonimide
(147 mg in 0.80 mL THF) was added over a 5 min period. The solution was slowly
warmed to room temperature and added to a saturated sodium bicarbonate solution.
It was extracted with ethyl acetate then dried over magnesium sulfate, filtered and
20   evaporated to dryness. The crude product was purified by preparative HPLC and.
isolated as the trifluoroacetate salt. The salt was neutralized by adding to a saturated
sodium bicarbonate solution and extracting with ethyl acetate. The purified product
was dried over magnesium sulfate, filtered and evaporated to dryness.

25   ## EXAMPLE 5-6
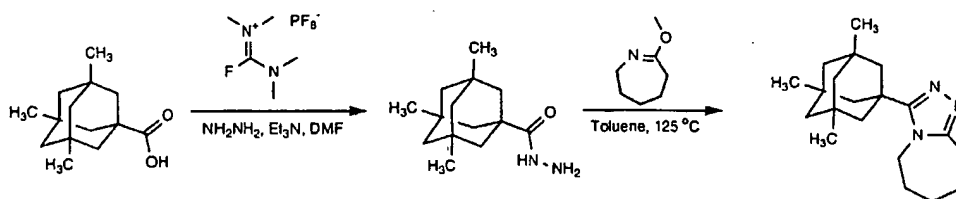
Preparation of 3-(3,5,8-trimethyladamantanyl)-4H,5H,6H,7H,8H-1,2,4-triazolo[4,3-
a]perhydroazepine (5-6)

         3,5,7-Trimethyladamantane-1-carboxylic acid was dissolved in DMF
(2 mL) and stirred at room temperature under nitrogen. Triethylamine (0.093 mL),
5   and fluoro-N,N,N'N'-tetramethylformamidinium hexafluorophosphate (88 mg) were
added. After 10 min, hydrazine hydrate (0.033 mL) was added and, after stirring for
15 min, water (2 mL) was added. The crude acyl hydrazide was collected by
filtration.

         3,5,7-Trimethyladamantane-1-carbohydrazide (26.2 mg), 1-aza-2-
10   methoxy-1-cycoheptene (16 µL) and anhydrous toluene (1 mL) were added to a small
vial and heated to 50 °C for 3 h. The solution was then heated to 120 °C for 4 h.
After cooling, the toluene was evaporated and the product was purified by column
chromatography (silica gel, 100% Ethyl acetate → 10% methanol/ethyl acetate →
10% methanol/CH$_2$Cl$_2$).

15

<p align="center">EXAMPLE 5-7</p>
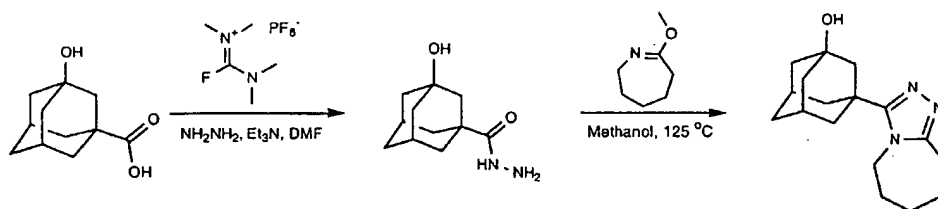
20   Preparation of 3-(4H,5H,6H,7H,8H-1,2,4-triazolo[4,5-a]perhydroazepin-3-
yl)adamantan-1-ol (5-7)

         3-Hydroxyadamantane-1-carboxylic acid was dissolved in DMF (3
mL) and stirred at room temperature under nitrogen. Triethylamine (0.33 mL), and
fluoro-N,N,N'N'-tetramethylformamidinium hexafluorophosphate (296 mg) were
25   added. After 10 min, hydrazine hydrate (0.114 mL) was added and after stirring for
15 min the reaction was evaporated to dryness. The crude 3-hydroxyadamantane-1-
carbohydrazide, 1-aza-2-methoxy-1-cycoheptene (0.2 mL) and anhydrous methanol (6
mL) were added to a small flask and heated to 50 °C for 3 h. The solution was then
heated to 70 °C for 24 h. After cooling, the methanol was evaporated and the product
30   was purified by column chromatography (silica gel, 100% Ethyl acetate → 10%
methanol/ ethyl acetate → 10% methanol/CH$_2$Cl$_2$).

EXAMPLE 5-8
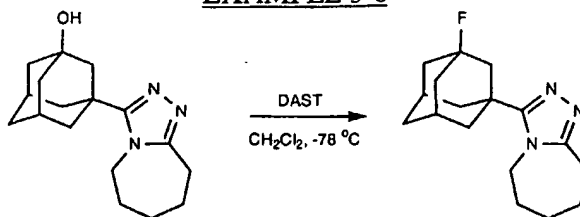
5   Preparation of 3-(3-fluoroadamantanyl)-4H,5H,6H,7H,8H-1,2,4-triazolo[4,3-
     a]perhydroazepine (5-8)

       The compound of Example 5-7 (18 mg), was dissolved in methylene
chloride (2 mL) and cooled to –78 °C while stirring under nitrogen.
(Diethylamino)sulfur trifluoride (9.1 µL) was added and the reaction was allowed to

10   slowly warm to 0 °C. The reaction was added to saturated sodium bicarbonate
solution and extracted with methylene chloride. The organic solution was dried over
magnesium sulfate, filtered and evaporated to dryness. The product was purified by
column chromatography (silica gel, 100% Ethyl acetate → 10% methanol/ ethyl
acetate → 10% methanol/CH_2Cl_2).

15

EXAMPLE 5-9
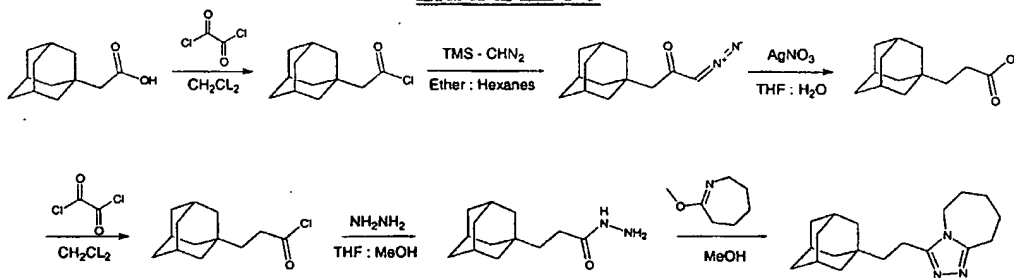
Preparation of 3-(2-adamantanylethyl)-4H,5H,6H,7H,8H-1,2,4-triazolo[4,3-
20   a]perhydroazepine (5-9)

       Adamantaneacetic acid (0.4814 g) was dissolved in dry methylene
chloride and stirred at room temperature under nitrogen. Oxalyl chloride (0.423 mL)
was added and the solution was stirred for 2 h whereupon the volatiles were removed.
The resulting acid chloride was dissolved in dry diethyl ether and stirred under

25   nitrogen at room temperature. Trimethylsilyldiazomethane (1.7 mL, 2M in hexanes)

was added and the reaction was stirred 36 h. The solution was washed with saturated
aqueous sodium bicarbonate and extracted with ether (2X) . The ether layers were
combined, dried with magnesium sulfate and the solvent removed. The product was
purified by silica gel chromatography (10% ethyl acetate/Hexane to 20% ethyl
5   acetate/Hexane) to give 72.3 mg of the desired diazoketone.

The diazoketone was dissolved in THF (3 mL) and water (6 mL) and
stirred at room temperature. Silver nitrate (67 mg) was added and the reaction was
stirred in the dark for 15 h. The solution was added to additional water (10 mL) and
extracted with ethyl acetate (2X). The organic layers were combined, dried
10   (magnesium sulfate), filtered and the solvent evaporated. The product was purified by
silica gel chromatography (20:79:1 ethyl acetate:hexanes:acetic acid → 30:69:1 ethyl
acetate:hexanes:acetic acid → 50:49:1 ethyl acetate:hexanes:acetic acid) and provided
45 mg of the desired carboxylic acid.
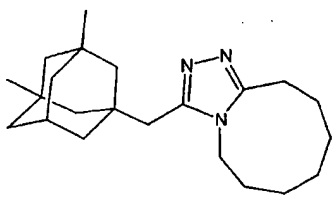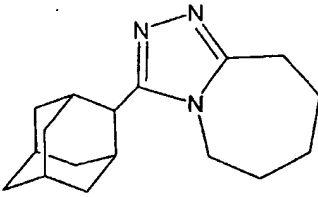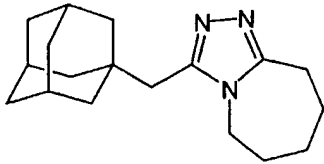
The carboxylic acid (45 mg) was dissolved in dry methylene chloride
15   and under nitrogen stirred at room temperature. Oxalyl chloride (0.100 mL) was
added and the solution was stirred for 2 h whereupon the product was dried *in vacuo*.
The acid choride was dissolved in tetrahydrofuran (2 mL) and rapidly added to a
solution of hydrazine (1 mL), THF (1mL) and methanol (1 mL) which was stirred
under nitrogen and cooed to 0 °C. After slowly warming to room temperature the
20   reaction was dried *in vacuo*. The crude product was added to ethyl acetate and
extracted with saturated sodium chloride solution containing about 2% sodium
hydroxide. After extraction (2X), the organic layers were combined, dried
(magnesium sulfate), filtered and the solvent evaporated. After thorough drying, the
crude acyl hydrazide was dissolved in dry methanol (5 mL). 1-Aza-2-methoxy-1-
25   cycoheptene (48 μL) was added and the solution was stirred at 50 °C overnight and
70 °C for 48 h. The solution was evaporated to dryness and purified by preparative
HPLC. The resulting trifluoroacetate salt was neutralized by adding to a saturated
sodium bicarbonate solution and extracting with ethyl acetate. The purified product
was dried over magnesium sulfate, filtered and evaporated to dryness.
30
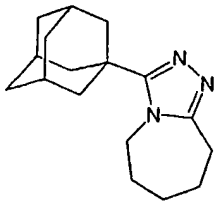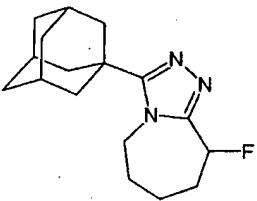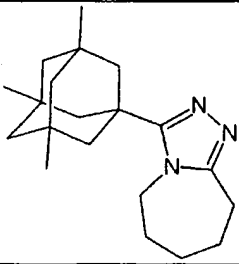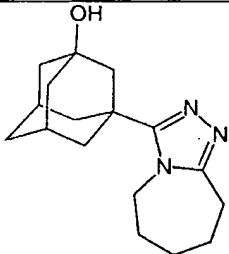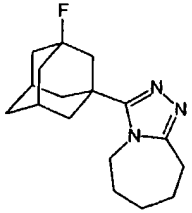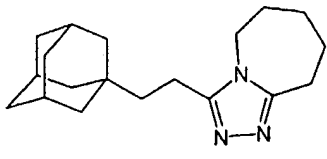
Preparative LC Method:

Column:	YMC – PACK ODS, 100 mm X 20 mm, 5.0 μm
Eluent A:	0.05% TFA in Water
35 Eluent B:	0.05% TFA in Acetonitrile

Pre-inject Equilibration: 1.0 min
Post-Inject Hold: 0.5 min
Gradient: 10 % B to 100 % B : between 10 and 20 min, hold at
                              100 % B for an additional 1.0 min, ramp back from
                              100% B to 10 % B in 0.5 min
Flow: 20 mL/min
Column Temperature: ambient
Injection amount: 5.0 mL
Detection: photodiode array

Ex.	Structure	Name	Retention Time (min)	MS ESI (m/z)
5-1		3-[(3,5-dimethyl-1-adamantyl)methyl]-6,7,8,9,10,11-hexahydro-5H-[1,2,4]triazolo[4,3-a]azonine	3.34	342.4
5-2		3-(2-adamantyl)-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepine	2.46	272.3
5-3		3-(1-adamantylmethyl)-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepine trifluoroacetate salt	2.54	286.4

5-4		3-(1-adamantyl)-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepine	2.05	272.2
5-5		3-(1-adamantyl)-9-fluoro-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepine	2.23	290.2
5-6		3-(3,5,7-trimethyl-1-adamantyl)-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepine	2.82	314.3
5-7		3-(6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepin-3-yl)adamantan-1-ol	1.22	288.2
5-8		3-(3-fluoro-1-adamantyl)-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepine	1.84	290.2
5-9		3-[2-(1-adamantyl)ethyl]-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepine	2.66	300.3

Analytical LC Method:

	Column:	Waters- XTerra C18, 5 μm, 4.6x50 mm
	Eluent A:	0.6% TFA in Water
5	Eluent B:	0.5 % TFA in Acetonitrile
	Gradient:	10 % B to 90 % B in 4.5 min, hold for 0.5 min, ramp
		back to 105 % B in 0.5 min
	Flow:	2.5 mL/min (going into the MS=250 μl)
	Column Temperature:	30°C
10	Injection amount:	10 μl of undiluted crude reaction mixture.
	Detection:	DAD: 190-600 nm.
		MS: API-ES positive ionization mode,
		Variable mass scan range:
		LC1-XLo = 50-500 amu
15		LC1-Low= 150-750 amu
		LC1-Med= 300-1000 amu
		LC1-High=500-2000 amu

EXAMPLE 5-10

20

Preparation of 3-(3-bromoadamantanyl)-4H,5H,6H,7H,8H-1,2,4-triazolo[4,3-
a]perhydroazepine (5-10)

25   900 mg of 3-Bromoadamantanecarboxylic acid was added to a dry
flask and dissolved in 10 mL dry methylene chloride. 1.22 mL of Oxalyl chloride was

added and the solution was stirred at room temperature for 1 h whereupon the solution was evaporated to dryness. The crude acid chloride was dissolved in 10 mL DMF and added dropwise to a stirring solution of DMF (10 mL) and hydrazine (1.04 mL) at room temperature. Water was added and the solution was filtered. The filtrate was extracted with methylene chloride and the solid product was purified by silica gel chromatography (5% methanol in methylene chloride) to give 489 mg of the desired 3-bromoadamantanecarbohydrazide.

To a dry flask was added 480 mg 3-bromoadamantanecarbohydrazide and 12 mL anhydrous methanol. After 5 min, the imino ether (0.504 mL) was added dropwise. The solution was stirred under nitrogen at room temperature for 40 min, warmed to 41 °C for two h, and refluxed for 24 h. The solution was cooled and evaporated to dryness. Purification with silica gel (50/49.9/0.1, ethyl acetate/methylene chloride/ acetic acid) provided 559 mg of the title compound.

EXAMPLE 5-11

5-10   AlBr₃ / benzene   5-11
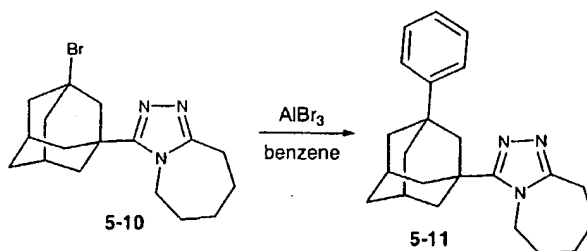
Preparation of 3-(3-phenyladamantanyl)-4H,5H,6H,7H,8H-1,2,4-triazolo[4,3-a]perhydroazepine (5-11)

65.4 mg of Aluminum tribromide was placed in a dry 10-mL flask. 0.5 mL dry benzene was added and the mixture was cooled in an ice bath. 25 mg of compound **5-10** was rapidly added and the solution was slowly warmed to room temperature and stirred for an additional 18 h. The reaction was quenched with ice and acidified with 2N HCl. The organic layer was separated and washed with water (2X) and brine. The organic solution was dried over magnesium sulfate, filtered and evaporated. The crude product was purified by preparative HPLC to provide **5-11** as its trifluoroacetate salt.

Synthesis of Compounds 5-12, 5-13 and 5-14. General Scheme:
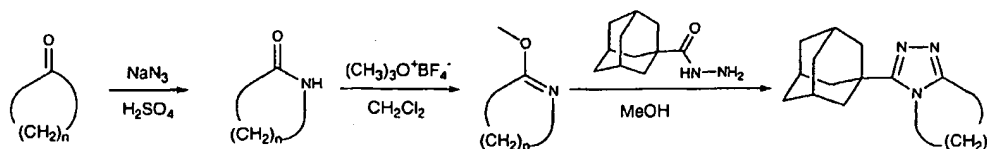
5

EXAMPLE 5-12
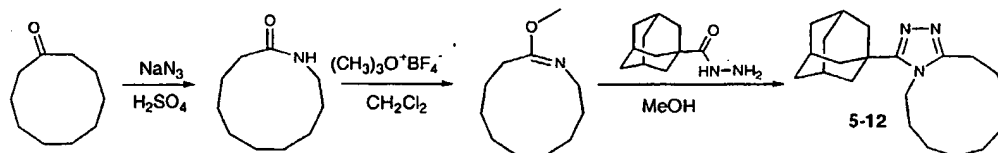
Preparation of 3-adamantanyl-4,5,6,7,8,9,10,11,12,3a-decahydro-1,2,4-triazolo[4,3-
10   a][11]annulene (5-12)

Cyclodecanone (n=6) (1.0 g) in 10 mL concentrated sulfuric acid was
cooled to 0 °C and 0.54 g of sodium azide was added. The reaction continued to stir
at 0 °C for 1 h and warmed to room temperature where it was stirred for two h. The
solution was diluted with cold water and treated with cold 10% NaOH solution until
15   pH =9. Extraction with ether (2X), drying over magnesium sulfate and evaporation
of solvent provided 1.23 g of 2-azacycloundecanone.

2-Azacycloundecanone (0.87 g) was dissolved in 20 mL methylene
chloride and stirred at room temperature under nitrogen. 1.5 g Trimethyloxonium
tetrafluoroborate was added and the reaction stirred overnight. The mixture was
20   added to saturated aqueous sodium bicarbonate and extracted with methylene chloride
(2X). The combined organic layers were washed with brine, dried over magnesium
sulfate, and the solvent evaporated to provide crude 2-methoxyazacyclododec-1-ene.

Adamantanecarbohydrazide (45 mg) was added to a small dry flask
and dissolved in 3mL dry methanol. 63.7 mg of 2-methoxyazacyclododec-1-ene was
25   added and the mixture was refluxed at 70 °C overnight. The methanol was removed
by evaporation and 3 mL toluene added. This mixture was refluxed 24 h at 122 °C.
The toluene was evaporated and the resulting solid was purified by preparative HPLC
(100% gradient/12min) to provide 5-12 as the trifluoroacetate salt.

The reaction sequence was repeated in similar fashion starting with cycloundecanone and cyclononanone to prepare 3-adamantanyl-

5   4,5,6,7,8,9,10,11,12,13,3a-undecahydro-1,2,4-triazolo[4,3-a][12]annulene (**5-13**) and 3-adamantanyl-4H,5H,6H,7H,8H,9H,10H,11H-1,2,4-triazolo[4,3-a]perhydroazepine (**5-14**), respectively.

EXAMPLE 5-15

10
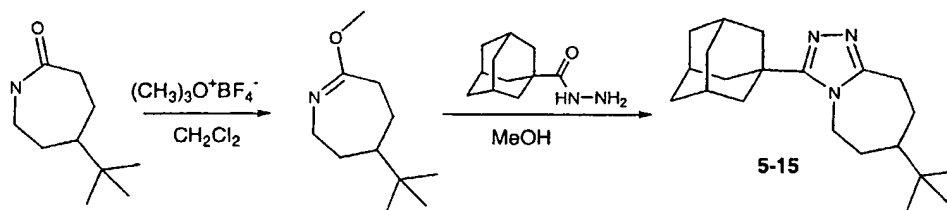
Preparation of 3-Adamantanyl-6-(tert-butyl)-4H,5H,6H,7H,8H-1,2,4-triazolo[4,3-a]perhydroazepine (5-15)

15   5-*tert*-Butylazocan-2-one (30 mg) was dissolved in 2 mL methylene chloride and stirred at room temperature under nitrogen. 31.3 g Trimethyloxonium tetrafluoroborate was added and the reaction stirred overnight. The mixture was added to saturated aqueous sodium bicarbonate and extracted with methylene chloride (2X). The combined organic layers were washed with brine, dried over magnesium

20   sulfate, and the solvent evaporated to provide crude 5-*tert*-butyl-8-methoxy-2,3,4,5,6,7-hexahydroazocine.

Adamantanecarbohydrazide (30 mg) was added to a small dry flask and dissolved in 3 mL dry methanol. The crude 5-*tert*-butyl-8-methoxy-2,3,4,5,6,7-hexahydroazocine was added and the mixture was refluxed at 70 °C overnight. The

25   methanol was removed by evaporation and 3 mL toluene added. This mixture was refluxed 24 h at 122 °C. The toluene was evaporated and the resulting solid was purified by preparative HPLC (100% gradient/12min) to provide **5-15** as the trifluoroacetate salt.

30   The reaction sequence was carried out in a similar manner to prepare the compounds of Examples **5-16** through **5-20** listed in the table below:

EXAMPLE 5-21
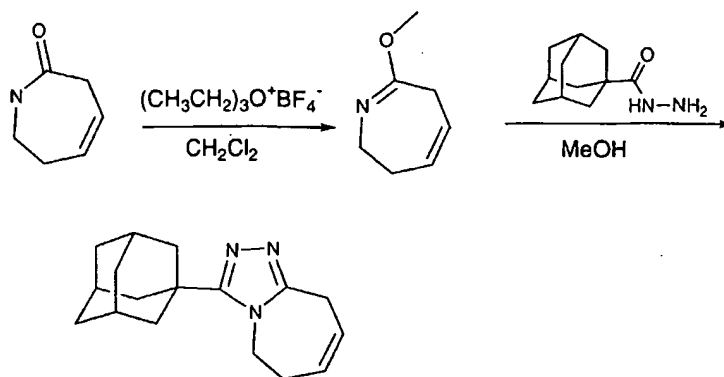
5   Preparation of 3-adamantanyl-4H,5H,8H-1,2,4-triazolo[4,3-a]azepine (5-21)

3,6,7,8-Tetrahydroazocin-2(1*H*)-one (75 mg) was dissolved in 1 mL
methylene chloride and stirred at room temperature under nitrogen. 0.81 mL
triethyloxonium tetrafluoroborate solution in methylene chloride (1.0M) was added
and the reaction stirred for 3 h. An additional 0.9 mL triethyloxonium
10   tetrafluoroborate solution was added. After stirring overnight, diisopropylethylamine
(0.14 mL) was added along with adamantanecarbohydrazide (130 mg) and dry
methanol (2 mL). The mixture was stirred at 45°C overnight and then refluxed for 24
h at 75 °C. The solvent was evaporated and the resulting solid was purified by
preparative HPLC (100% gradient/12min) to provide **5-21** as the trifluoroacetate salt.
15

The reaction sequence was repeated in similar fashion to prepare the
compounds of Examples **5-22** and **5-23**.

Preparative LC Method:
20

Column:	YMC – PACK ODS, 100 mm X 20 mm, 5.0 µm
Eluent A:	0.05% TFA in Water
Eluent B:	0.05% TFA in Acetonitrile
Pre-inject Equilibration:	1.0 min
25 Post-Inject Hold:	0.5 min
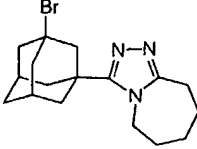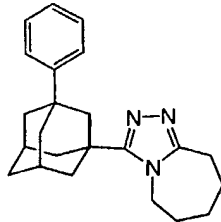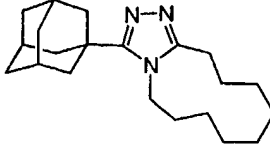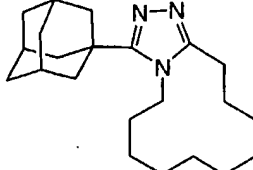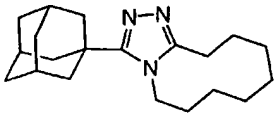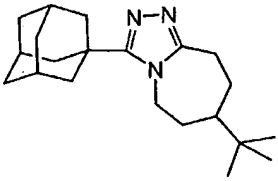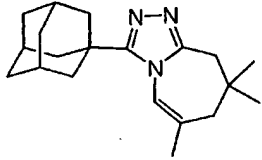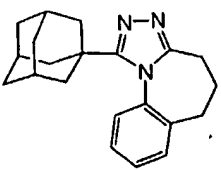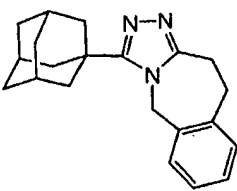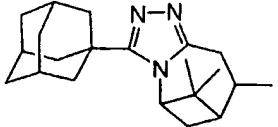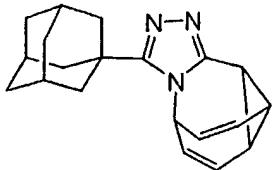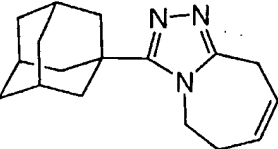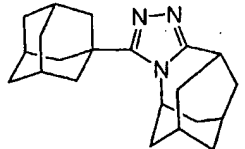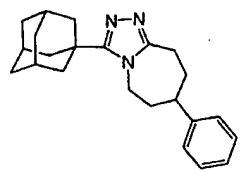
Gradient:	10 % B to 100 % B : between 10 and 20 min, hold at 100 % B for an additional 1.0 min, ramp back from 100% B to 10 % B in 0.5 min
Flow:	20 mL/min
Column Temperature:	ambient
Injection amount:	5.0 mL
Detection:	photodiode array

Table

Ex.	Structure	Name	Retention Time (min)	MS ESI (m/z)
5-10		3-(3-bromo-1-adamantyl)-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepine	2.42	350.3
5-11		3-(3-phenyl-1-adamantyl)-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepine trifluoroacetate salt	2.96	348.3
5-12		3-(1-adamantyl)-6,7,8,9,10,11,12,13-octahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azacycloundecine trifluoroacetate salt	3.09	328.3
5-13		3-(1-adamantyl)-5,6,7,8,9,10,11,12,13,14-decahydro[1,2,4]tri	3.28	342.3

		azolo[4,3-*a*]azacyclododecine trifluoroacetate salt		
<u>5-14</u>		3-(1-adamantyl)-5,6,7,8,9,10,11,12-octahydro[1,2,4]triazolo[4,3-*a*]azecine trifluoroacetate salt	2.88	314.3
<u>5-15</u>		3-(1-adamantyl)-7-*tert*-butyl-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepine trifluoroacetate salt	2.88	328.3
<u>5-16</u>		3-(1-adamantyl)-6,8,8-trimethyl-8,9-dihydro-7*H*-[1,2,4]triazolo[4,3-*a*]azepine trifluoroacetate salt	2.85	312.3
<u>5-17</u>		1-(1-adamantyl)-5,6-dihydro-4*H*-[1,2,4]triazolo[4,3-*a*][1]benzazepine trifluoroacetate salt	2.69	320.3
<u>5-18</u>		3-(1-adamantyl)-10,11-dihydro-5*H*-[1,2,4]triazolo[4,3-*b*][2]benzazepine trifluoroacetate salt	2.53	320.3

5-19		3-(1-adamantyl)-6,6,8-trimethyl-6,7,8,9-tetrahydro-5H-5,7-methano[1,2,4]triazolo[4,3-a]azepine trifluoroacetate salt	2.69	326.3
5-20		3-(1-adamantyl)-5,7a,8,8a-tetrahydro-5,8-ethenocyclopropa[c][1,2,4]triazolo[4,3-a]azepine trifluoroacetate salt	2.48	306.3
5-21		3-(1-adamantyl)-6,9-dihydro-5H-[1,2,4]triazolo[4,3-a]azepine trifluoroacetate salt	2.05	270.2
5-22		3-(1-adamantyl)-6,7,8,9,10,11-hexahydro-5H-5,9:7,11-dimethano[1,2,4]triazolo[4,3-a]azonine trifluoroacetate salt	2.40	324.3
5-23		3-(1-adamantyl)-7-phenyl-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepine trifluoroacetate salt	2.72	348.2

Analytical LC Method:

Column:	Waters- XTerra C18, 5 μm, 4.6x50 mm
Eluent A:	0.6% TFA in Water
5 Eluent B:	0.5 % TFA in Acetonitrile
Gradient:	10 % B to 90 % B in 4.5 min, hold for 0.5 min, ramp
	back to 105 % B in 0.5 min
Flow:	2.5 mL/min (going into the MS=250 μl)
Column Temperature:	30°C
10 Injection amount:	10 μl of undiluted crude reaction mixture.
Detection:	DAD: 190-600 nm
	MS: API-ES positive ionization mode,
	Variable mass scan range:
	LC1-XLo = 50-500 amu
15	LC1-Low= 150-750 amu
	LC1-Med= 300-1000 amu
	LC1-High=500-2000 amu

EXAMPLE 6-1

20

Preparation of 3-{[2-(4-chlorophenyl)adamantan-2-yl]methyl}-4H-1,2,4-triazole (6-1)

25 a) Preparation of 2-[2-(4-chlorophenyl)adamantan-2-yl] acetamide (6-1a)

To a solution of 2-[2-(4-chlorophenyl)adamantan-2-yl] acetic acid (100 mg, 0.33 mmol) in 4 mL N,N-dimethylformamide (DMF) were added sequentially ammonium chloride (88 mg, 1.6 mmol), 1-hydroxybenzotriazole hydrate (HOBt, 67 mg, 0.49 mmol), N, N-diisopropylethylamine (575 μL, 3.3 mmol), and 1-ethyl-3-(3-

dimethylaminopropyl)carbodiimide hydrochloride (EDC, 95 mg, 0.49 mmol). The
mixture was stirred at room temperature under nitrogen for 2 h, then added to a
separatory funnel containing 50 mL of ethyl acetate and aqueous hydrochloric acid
(HCl, 1N). The layers were separated and the organic layer was washed sequentially
5   with aqueous N HCl , saturated aqueous sodium bicarbonate, and brine. The organic
layer was dried over anhydrous sodium sulfate and evaporated *in vacuo* to yield 82 mg
of title compound as a white powder which was used without purification.

b) Preparation of methyl 2-[2-(4-chlorophenyl)-2-adamantyl]ethanimidoate (6-1b)
10   A solution of **6-1a** (30 mg, 0.1 mmol) in 0.5 mL of anhydrous
methylene chloride was treated with trimethyloxonium tetrafloroborate (30 mg, 0.2
mmol). The mixture was stirred under nitrogen for 18 h, then added to a separatory
funnel containing 25 mL of methylene chloride and saturated aqueous sodium
bicarbonate solution. The layers were mixed and separated and the organic layer was
15   dried over anhydrous sodium sulfate and evaporated *in vacuo* to yield 32 mg of title
compound, which was used without purification.

c) Preparation of 3-{[2-(4-chlorophenyl)adamantan-2-yl]methyl}-4H-1,2,4-triazole
                A solution of **6-1b** (32 mg, 0.1 mmol) and formic hydrazide (9 mg,
20   0.15 mmol) in anhydrous toluene was refluxed under nitrogen for 18 h. The mixture
was evaporated to dryness and the residue purified by reverse phase HPLC to give
title compound as a white powder.

<div align="center">

EXAMPLE 6-2

</div>

25
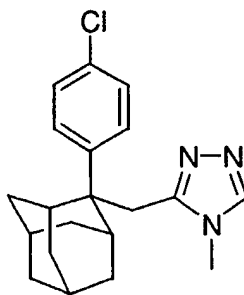
Preparation of 3-{[2-(4-chlorophenyl)adamantan-2-yl]methyl}-4-methyl-1,2,4-triazole
(6-2)

a) Preparation of 2-[2-(4-chlorophenyl)adamantan-2-yl]-*N*-methylacetamide (6-2a)

        The title compound was prepared by an identical procedure to the one described for example **6-1a** using methylamine hydrochloride.

b) Preparation of 2-[2-(4-chlorophenyl)adamantan-2-yl]-*N*-methylethanethioamide (6-2b)

        A solution of **6-2a** (12 mg, 0.036 mmol) and Lawesson's reagent (22 mg, 0.054 mmol) in 0.5 mL of toluene was refluxed under nitrogen for 2 h. The mixture was added to a separatory funnel containing ethyl acetate and saturated aqueous solution of ammonium chloride. The organic layer was washed with saturated aqueous sodium bicarbonate and brine, dried over anhydrous sodium sulfate, and evaporated to dryness to yield 24 mg of crude mixture containing the title compound which was used without purification.

c) Preparation of 3-{[2-(4-chlorophenyl)adamantan-2-yl]methyl}-4-methyl-1,2,4-triazole (6-2)

        To a crude mixture containing **6-2b** (24 mg) dissolved in 4:1 toluene:butanol (1 mL) were added sequentially formic hydrazide (20 mg, 0.3 mmol) and silver trifloromethanesulfonate (40 mg, 0.15 mmol). The mixture was stirred at reflux under nitrogen for 2 h, then filtered through celite and washed with methanol (30 mL). The filtrate was evaporated to dryness and purified by reverse phase HPLC to yield title compound as the TFA salt.

Table 1: Analytical data for examples 6-1 and 6-2.

Compound	Retention time (min)	MS ESI (*m/z*)
6-1	1.84	328
6-2	1.84	342

HPLC Conditions:

Analytical LC Method:

5

Column:	MetaChem Polaris C-18A, 30 mm X 4.6 mm, 5.0 μm
Eluent A:	0.1% TFA in Water
Eluent B:	0.1 % TFA in Acetonitrile
Gradient:	5 % B to 95 % B in 3.3 min, ramp back to 5 % B in 0.3 min
Flow:	2.5 mL/min
Column Temperature:	50 °C
Injection amount:	5 μl of undiluted crude reaction mixture.
Detection:	DAD: 190-600 nm
	MS: API-ES ionization mode, mass scan range (100-600)
	ELSD: Light Scattering Detector

10

15

Preparative LC Method:

20

Column:	YMC – PACK ODS, 100 mm X 20 mm, 5.0 μm
Eluent A:	0.1% TFA in Water
Eluent B:	0.1 % TFA in Acetonitrile
Pre-inject Equilibration:	1.0 min
Post-Inject Hold:	1.0 min
Gradient:	10 % B to 100 % B in 7.5 min, hold at 100 % B for an additional 1.0 min, ramp back from 100% B o 10 % B in 1.5 min
Flow:	20 mL/min
Column Temperature:	ambient
Injection amount:	2.0 mL of crude reaction mixture.
Detection:	UV at 220 nm.

25

30

EXAMPLE 7

Preparation of 3-adamantanyl-4H,5H,8H,9H-1,2,4-triazolo[4,3-a]azocine

5·
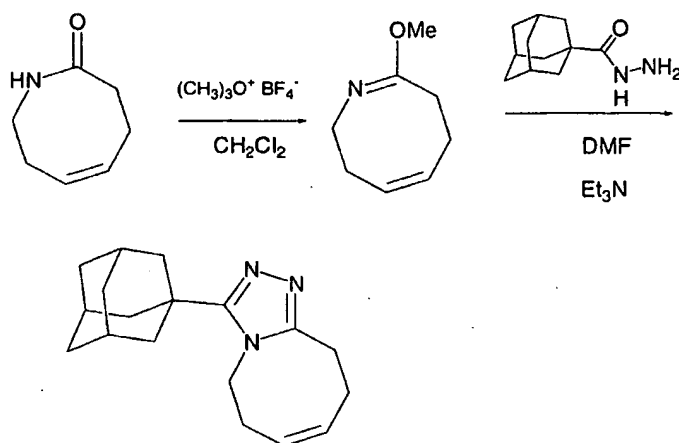
To a sample of 103 mg (0.822 mmol) of 1H,3H,4H,7H,8H-azocin-2-one in 5 mL of dichloromethane was added 183 mg (1.234 mmol) of trimethyloxonium tetrafluoroborate. The reaction was stirred at room temperature for

10   16 h, after which time it was diluted with 15 mL of methylene chloride and extracted twice with 5 mL of saturated aqueous NaHCO₃ and once with 5 mL of brine. The organic layer was dried over MgSO₄, filtered, and the concentrated under reduced pressure. The 8-methoxy-2H,3H,6H,7H-azocine thus produced (92 mg) was used without purification in the next reaction. ^{1}H NMR (500 MHz, CDCl₃): δ 5.82 (m,

15   1H), 5.69 (m, 1H), 4.22 (s, 3H), 4.04 (q, 2H, J = 6 Hz), 3.03 (t, 2H, J = 6 Hz), 2.73 (br apparent q, 2H, J = 6Hz), 2.63 (apparent q, 2H, J = 6Hz).

To a sample of 55 mg (0.395 mmol) of 8-methoxy-2H,3H,6H,7H-azocine in 3 mL of N,N-dimethylformamide was added 194 mg of adamantyl hydrazide (0.593 mmol) and 0.256 mL (1.976 mmol) of triethylamine. The reaction

20   was heated in a sealed tube at 100 °C for 1 h. The solvent was removed under vacuum, and the residue was chromatographed on silica gel eluting first with ethyl acetate, then with methylene chloride, 2% methanol in methylene chloride, and finally 5% methanol in methylene chloride at which time the desired product eluted from the column. This afforded 12.2 mg of the desired triazole. ^{1}H NMR (500 MHz, CDCl₃):

δ 5.82 (m, 1H), 5.50 (m, 1H), 4.62 (br t, 2H, J = 6.9 Hz), 3.69 (br t, 2H, J = 6.9 Hz), 2.85 (br apparent q, 2H, J = 5.7 Hz, 6.7 Hz), 2.72 (br apparent q, 2H, J = 6.7 Hz, 6.9 Hz), 2.18 (br s, 3H), 2.13 (br s, 6H), 1.82 (AB pattern, 6H, J = 15.8 Hz, J = 12.3 Hz). Mass spectrum (electrospray): 284 (M + 1).

EXAMPLE OF A PHARMACEUTICAL FORMULATION

As a specific embodiment of an oral composition of a compound of the present invention, 50 mg of any of Examples 1 is formulated with sufficient finely divided lactose to provide a total amount of 580 to 590 mg to fill a size O hard gelatin capsule.

While the invention has been described and illustrated in reference to specific embodiments thereof, those skilled in the art will appreciate that various changes, modifications, and substitutions can be made therein without departing from the spirit and scope of the invention. For example, effective dosages other than the preferred doses as set forth hereinabove may be applicable as a consequence of variations in the responsiveness of the human being treated for a particular condition. Likewise, the pharmacologic response observed may vary according to and depending upon the particular active compound selected or whether there are present pharmaceutical carriers, as well as the type of formulation and mode of administration employed, and such expected variations or differences in the results are contemplated in accordance with the objects and practices of the present invention. It is intended therefore that the invention be limited only by the scope of the claims which follow and that such claims be interpreted as broadly as is reasonable.

WHAT IS CLAIMED IS:

1.   A compound of structural formula I, or a pharmaceutically acceptable salt or prodrug thereof,

$$R^1X-\underset{N-N}{\overset{\overset{\displaystyle WR^2}{|}}{\overset{\displaystyle N}{<}}}-ZR^3$$

(I)
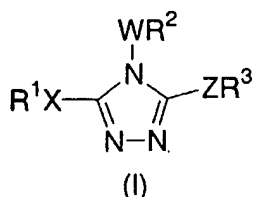
5

wherein:

R^1 is adamantyl, unsubstituted or substituted with one to five substituents independently selected from halogen, OCH_3, OCF_3, CH_3, CF_3, and phenyl, wherein said phenyl is unsubstituted or substituted with one to three halogens;

10

W is selected from the group consisting of NR^a and a single bond;
X is selected from the group consisting of CH_2 and a single bond;

Z is selected from the group consisting of S and a single bond;

15   R^a is selected from the group consisting of hydrogen and C_{1-6} alkyl, wherein alkyl is unsubstituted or substituted with one to five fluorines;

R^2 is selected from the group consisting of
        hydrogen,
20   C_{1-10} alkyl, unsubstituted or substituted with one to six substituents independently selected from zero to five halogens and zero or one group selected from hydroxy and C_{1-3} alkoxy, said alkoxy group being unsubstituted or substituted with one to three halogens,
        C_{2-10} alkenyl, unsubstituted or substituted with one to six substituents
25   independently selected from zero to five halogens and zero or one group selected from hydroxy and C_{1-3} alkoxy, said alkoxy group being unsubstituted or substituted with one to three halogens,
        CH_2CO_2H,
        $CH_2CO_2C_{1-6}$ alkyl,

CH2CONHR^a,

(CH2)0-2C3-9 cycloalkyl,

(CH2)0-2C5-12 bicycloalkyl,

(CH2)0-2adamantyl, and

5   (CH2)0-2R;

wherein said C3-9 cycloalkyl and C5-12 bicycloalkyl optionally have one to two

double bonds, and said C3-9 cycloalkyl, C5-12 bicycloalkyl, and adamantyl are

unsubstituted or substituted with one to six substituents independently selected from

(a) zero to five halogens, CH3, CF3, OCH3, and OCF3, and (b) zero or one phenyl,

10   said phenyl being unsubstituted or substituted with one to four groups independently

selected from halogen, OCH3, OCF3, CH3, and CF3;

R^3 is selected from the group consisting of

          hydrogen,

15   C1-10 alkyl, unsubstituted or substituted with one to six substituents

independently selected from zero to five halogens and zero or one group selected from

hydroxy and C1-3 alkoxy, said alkoxy group being unsubstituted or substituted with

one to three halogens,

          C2-10 alkenyl, unsubstituted or substituted with one to six substituents

20   independently selected from zero to five halogens and zero or one group selected from

hydroxy and C1-3 alkoxy, said alkoxy group being unsubstituted or substituted with

one to three halogens,

          YC3-9 cycloalkyl,

          YC5-12 bicycloalkyl,

25   Yadamantyl, and

          YR;

wherein said C3-9 cycloalkyl and C5-12 bicycloalkyl optionally have one to two

double bonds, and said C3-9 cycloalkyl, C5-12 bicycloalkyl, and adamantyl are

unsubstituted or substituted with one to six substituents independently selected from

30   (a) zero to five halogens, CH3, CF3, OCH3, and OCF3, and (b) zero or one phenyl,

said phenyl being unsubstituted or substituted with one to four groups independently

selected from halogen, OCH3, OCF3, CH3, and CF3;

R is selected from the group consisting of benzodioxolane, furan, tetrahydrofuran, thiophene, tetrahydrothiophene, dihydropyran, tetrahydropyran, pyridine, piperidine, benzofuran, dihydrobenzofuran, benzothiophene, dihydrobenzothiophene, indole, dihydroindole, indene, indane, 1,3-dioxolane, 1,3-dioxane, phenyl, and naphthyl;

5   wherein R is unsubstituted or substituted with one to four groups independently selected from halogen, C_{1-4} alkylthio, C_{1-4} alkylsulfinyl, C_{1-4} alkylsulfonyl, C_{2-4} alkenylsulfonyl, CN, OH, OCH_3, OCF_3, and C_{1-4} alkyl, said C_{1-4} alkyl being unsubstituted or substituted with one to five halogens or one substituent selected from OH and C_{1-3} alkoxy; and

10

Y is selected from $(CH_2)_{0-2}$ and (-HC=CH-);

or alternatively R^2 and R^3 taken together form a bridging group R^4, providing a compound of structural formula Ia:

$$\underset{\displaystyle \text{Ia}}{\chemfig{}}$$

W—R⁴ / N / Z over R¹X— / N—N

15
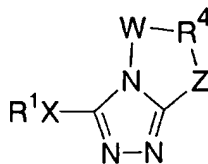
wherein R^4 is

a C_{2-8} alkylene group, optionally containing one heteroatom selected from O and NR^b between two adjacent carbon atoms of said C_{2-8} alkylene group, optionally

20   containing one to two carbon-carbon double bonds when R^4 is a C_{3-8} alkylene group, and optionally also comprising a carbon-carbon single bond connecting two non-adjacent carbon atoms of said C_{2-8} alkylene group, or

a C_{4-8} cycloalkyl group;

wherein R^b is selected from the group consisting of hydrogen and C_{1-6} alkyl,

25   unsubstituted or substituted with one to six substituents independently selected from zero to five fluorines and zero or one phenyl, said phenyl being unsubstituted or substituted with one to three substituents independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3;

wherein R^4 is unsubstituted or substituted with one to five R^c substituents, wherein

30   each R^c is independently selected from halogen, OH, OCH_3, OCF_3, C_{1-6} alkyl,

C_{2-6} alkenyl, phenyl, biphenyl, C_{3-8} cycloalkyl, C_{1-6} alkyloxycarbonyl, an epoxide group bridging 2 adjacent carbons, and 1,3-dioxolanyl geminally disubstituted onto one carbon of R^4, wherein each C_{1-6} alkyl and C_{2-6} alkenyl is unsubstituted or substituted with one to five substituents independently selected from zero to three halogens and zero to two groups selected from phenyl, C_{1-6} alkyloxycarbonyl, 1,3-dioxolanyl geminally disubstituted onto one carbon, and CN, and wherein each phenyl, biphenyl, and C_{3-8} cycloalkyl, either as R^c or as a substituent on R^c, is unsubstituted or substituted with one to three groups independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3;

wherein R^4 optionally has a fused phenyl ring, a benzodioxinyl ring, or a dihydrobenzodioxinyl ring, said phenyl ring, benzodioxinyl ring, and dihydrobenzodioxinyl ring being unsubstituted or substituted with one to three substituents independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3; and

wherein R^4, including said optional fused phenyl ring, benzodioxinyl ring, or dihydrobenzodioxinyl ring and including all substituents on R^4 and said fused phenyl ring, benzodioxinyl ring, or dihydrobenzodioxinyl ring, has no more than 20 carbon atoms;

with the provisos that

(a) when X and W represent single bonds, Z is sulfur, R^1 is unsubstituted adamantyl, and R^3 is hydrogen, then R^2 is not hydrogen, methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, 2-butyl, tert-butyl, phenyl, CH_2phenyl, or cyclohexyl;

(b) when X and W represent single bonds, Z is sulfur, R^1 is unsubstituted adamantyl, and R^3 is ethyl, 3-propenyl, CH_2phenyl, 4-Cl-CH_2phenyl, or 4-NO_2-CH_2phenyl, then R^2 is not methyl;

(c) when X and W represent single bonds, Z is sulfur, R^1 is unsubstituted adamantyl, and R^3 is CH_2-(CO)-4-F-phenyl, then R^2 is not phenyl;

(d) when X and Z represent single bonds and R^1 is unsubstituted adamantyl, then R^2 and R^3 taken together cannnot form a C_{3-5} alkylene R^4 bridging group; and

(e) R^2 and R^3 are not both hydrogen.

2.   The compound of Claim 1 of structural formula I and not
structural formula Ia.

3.   The compound of Claim 1 of structural formula Ia and not

5   structural formula I.

4.   The compound of Claim 2 wherein
R^1 is adamantyl, unsubstituted or substituted with one to five substituents
independently selected from halogen, OCH_3, OCF_3, CH_3, CF_3, and phenyl, wherein

10   said phenyl is unsubstituted or substituted with one to three halogens;

X, W, and Z are single bonds;

R^2 is selected from the group consisting of

15   hydrogen,
          C_{1-6} alkyl, unsubstituted or substituted with one to four substituents
independently selected from zero to three halogens and zero or one group selected
from hydroxy and C_{1-3} alkoxy, said alkoxy group being unsubstituted or substituted
with one to three halogens,

20   C_{2-4} alkenyl, unsubstituted or substituted with one to four substituents
independently selected from zero to three halogens and zero or one group selected
from hydroxy and C_{1-3} alkoxy, said alkoxy group being unsubstituted or substituted
with one to three halogens,
          CH_2CO_2H,

25        $CH_2CO_2C_{1-3}$ alkyl,
          CH_2CONHR^a,
          $(CH_2)_{0-1}C_{3-6}$ cycloalkyl,
          $(CH_2)_{0-1}C_{4-6}$ cycloalkenyl,
          $(CH_2)_{0-1}$phenyl,

30        $(CH_2)_{0-1}$furyl,
     wherein cycloalkyl, cycloalkenyl, phenyl, and furyl are unsubstituted or substituted
with one to three groups independently selected from halogen, OCH_3, OCF_3, CH_3,
and CF_3;

R^a is selected from the group consisting of hydrogen and C_{1-6} alkyl, wherein alkyl is unsubstituted or substituted with one to five fluorines; and

R^3 is selected from the group consisting of

       hydrogen,

       C_{1-6} alkyl, unsubstituted or substituted with one to five halogens,

       C_{2-6} alkenyl, unsubstituted or substituted with one to five halogens,

       $(CH_2)_{0-1}C_{3-6}$ cycloalkyl, wherein cycloalkyl has one double bond and is unsubstituted or substituted with one to five substituents independently selected from the group consisting of (a) zero to five halogens and methyl and (b) zero or 1 phenyl,

       $(CH_2)_{0-1}$ adamantyl, unsubstituted or substituted with one to four substituents independently selected from halogen and methyl,

       $(CH_2)_{0-1}$ phenyl, unsubstituted or substituted with one to three substituents independently selected from methyl, cyano, hydroxymethyl, CF_3, OCF_3, hydroxy, OCH_3, halogen and $S(O)_{0-2}CH_3$, and

       YR, wherein Y is selected from the group consisting of CH_2, (-HC=CH-), and a bond, and R is selected from the group consisting of benzodioxolane, furan, thiophene, dihydrobenzofuran, tetrahydrofuran, tetrahydropyran, and indane, wherein R is unsubstituted or substituted with one to three halogens.

      5.   The compound of Claim 2 wherein

R^1 is adamantyl, unsubstituted or substituted with one to five substituents independently selected from halogen, OCH_3, OCF_3, CH_3, CF_3, and phenyl, wherein said phenyl is unsubstituted or substituted with one to three halogens;

X is a single bond;

Z is S;

WR^2 is selected from the group consisting of

       NH_2,

       hydrogen,

       C_{1-6} alkyl, unsubstituted or substituted with one to four substituents independently selected from zero to three halogens and zero or one group selected from hydroxy and methoxy,

C$_{2-4}$ alkenyl, unsubstituted or substituted with one to three halogens,

(CH$_2$)$_{0-1}$C$_{3-6}$ cycloalkyl, and

(CH$_2$)$_{0-2}$R, wherein R is selected from the group conssiting of phenyl, furan,

tetrahydrofuran, and piperidine; wherein R and cycloalkyl are unsubstituted or

substituted with one to three groups independently selected from halogen, OCH$_3$,

OCF$_3$, CH$_3$, and CF$_3$; and

R^3 is selected from the group consisting of

     hydrogen,

     C$_{1-6}$ alkyl, unsubstituted or substituted with hydroxy, methoxy, or one to five

halogens,

     C$_{2-6}$ alkenyl, unsubstituted or substituted with hydroxy, methoxy, or one to

five halogens,

     (CH$_2$)$_{0-2}$C$_{3-8}$ cycloalkyl, wherein cycloalkyl has one double bond and is

unsubstituted or substituted with one to four substituents independently selected from

the group consisting of (a) zero to three halogens and methyl and (b) zero or 1 phenyl,

and

     (CH$_2$)$_{0-1}$R, wherein R is selected from the group consisting of 1,3-dioxolane,

1,3-dioxane, phenyl, furan, and pyridine; wherein R is unsubstituted or substituted

with one to three groups independently selected from halogen, OCH$_3$, OCF$_3$, CH$_3$,

and CF$_3$.

     6.   The compound of Claim 3 wherein R^1 is adamantyl,

unsubstituted or substituted with one to five substituents independently selected from

halogen, OCH$_3$, OCF$_3$, CH$_3$, CF$_3$, and phenyl, wherein said phenyl is unsubstituted

or substituted with one to three halogens;

     X is a bond;

     Z is S;

     W is a bond or NH; and

     R^4 is a C$_{2-8}$ alkylene group, unsubstituted or substituted with one to

three substituents R^c, where each R^c is independently selected from halogen, CH$_3$,

CF$_3$, and phenyl, wherein phenyl is unsubstituted or substituted with one to three

substituents independently selected from halogen, CH$_3$, CF$_3$, OCH$_3$, and OCF$_3$.

7.   The compound of Claim 3 of structural formula Ia

(Ia)
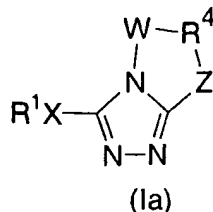
wherein:

R^1 is adamantyl, unsubstituted or substituted with one to five substituents

5   independently selected from halogen, OCH_3, OCF_3, CH_3, CF_3, and phenyl, wherein said phenyl is unsubstituted or substituted with one to three halogens;

X is selected from the group consisting of CH_2 and a single bond;

W and Z are single bonds; and

10

R^4 is

    a C_{3-8} alkylene group, optionally containing one heteroatom selected from O and NR^b between two adjacent carbon atoms of said C_{3-8} alkylene group, optionally containing one to two carbon-carbon double bonds when R^4 is a C_{3-8} alkylene group,

15   and optionally also comprising a carbon-carbon single bond connecting two non-adjacent carbon atoms of said C_{3-8} alkylene group, or

    a C_{4-8} cycloalkyl group;

wherein R^b is selected from the group consisting of hydrogen and C_{1-6} alkyl, unsubstituted or substituted with one to six substituents independently selected from

20   zero to five fluorines and zero to one phenyl, said phenyl being unsubstituted or substituted with one to three substituents independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3;

wherein R^4 is unsubstituted or substituted with one to five R^c substituents, wherein each R^c is independently selected from halogen, OH, OCH_3, OCF_3, C_{1-6} alkyl,

25   C_{2-6} alkenyl, phenyl, biphenyl, C_{3-8} cycloalkyl, C_{1-6} alkyloxycarbonyl, an epoxide group bridging 2 adjacent carbons, and 1,3-dioxolanyl geminally disubstituted onto one carbon of R^4, wherein each C_{1-6} alkyl and C_{2-6} alkenyl is unsubstituted or substituted with one to five substituents independently selected from zero to three halogens and zero to two groups selected from phenyl, C_{1-6} alkyloxycarbonyl, 1,3-

dioxolanyl geminally disubstituted onto one carbon, and CN, and wherein each phenyl, biphenyl, and C_{3-8} cycloalkyl, either as R^c or as a substituent on R^c, is unsubstituted or substituted with one to three groups independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3;

5

wherein R^4 optionally has a fused phenyl ring, a benzodioxinyl ring, or a dihydrobenzodioxinyl ring, said phenyl ring, benzodioxinyl ring, and dihydrobenzodioxinyl ring being unsubstituted or substituted with one to three substituents independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3; and

10

wherein R^4, including said optional fused phenyl ring, benzodioxinyl ring, or dihydrobenzodioxinyl ring and including all substituents on R^4 and said fused phenyl ring, benzodioxinyl ring, or dihydrobenzodioxinyl ring, has no more than 20 carbon atoms.

15

       8.   The compound of Claim 1 having formula I or formula Ia wherein Z is S and WR^2 is selected from NH_2 and R^2.

       9.   The compound of Claim 1 having formula I or formula Ia

20   wherein W and Z are single bonds.

       10.   The compound of Claim 1 selected from the group consisting of

25

and

;
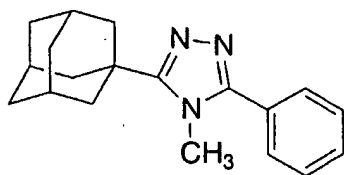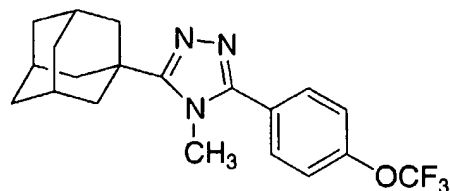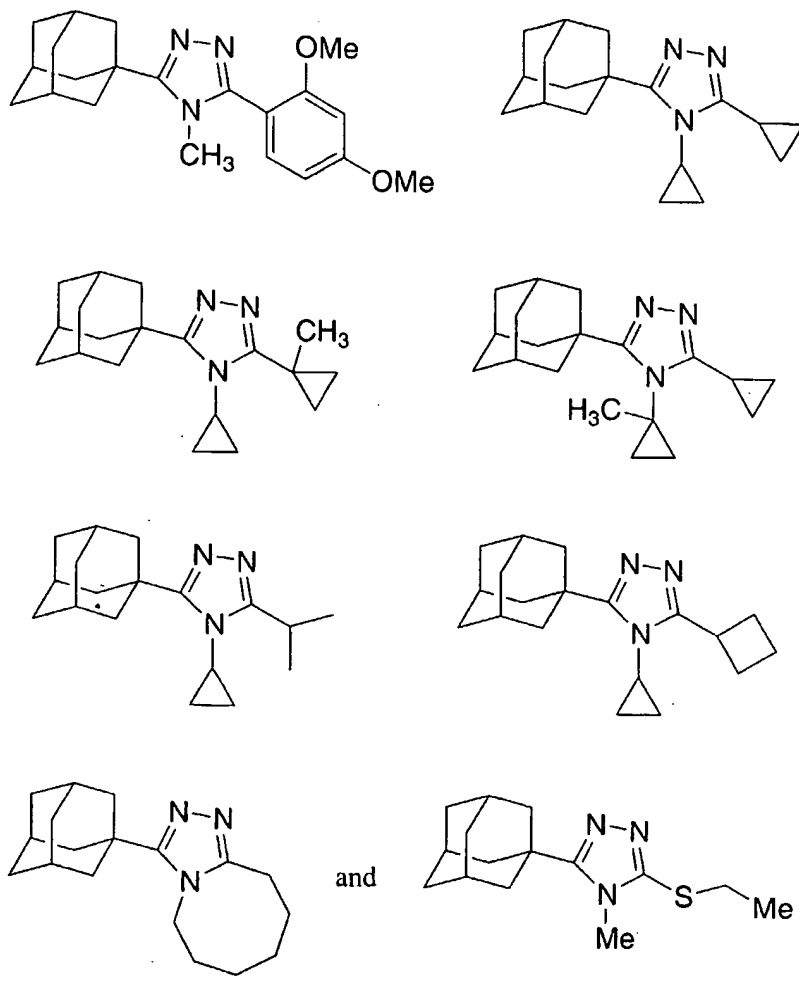
5   or a pharmaceutically acceptable salt or prodrug thereof.

11.   A pharmaceutical composition comprising a compound of
Claim 1 and a pharmaceutically acceptable carrier.

10   12.   A method for treating, controlling, or delaying the onset of non-
insulin dependent diabetes mellitus in a mammalian patient in need of such treatment
which comprises administering to said patient a therapeutically effective amount of a
compound of structural formula I, or a pharmaceutically acceptable salt thereof:
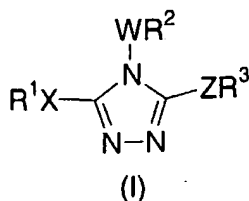
$$WR^2$$

$$R^1X\text{---}\underset{N\text{---}N}{\overset{N}{\diagdown}}\text{---}ZR^3$$

(I)

wherein:

R^1 is adamantyl, unsubstituted or substituted with one to five substituents
independently selected from halogen, OCH_3, OCF_3, CH_3, CF_3, and phenyl, wherein

5   said phenyl is unsubstituted or substituted with one to three halogens;

W is selected from the group consisting of NR^a and a single bond;
X is selected from the group consisting of CH_2 and a single bond;

Z is selected from the group consisting of S and a single bond;

10

R^a is selected from the group consisting of hydrogen and C_{1-6} alkyl, wherein alkyl is

unsubstituted or substituted with one to five fluorines;

R^2 is selected from the group consisting of

15   hydrogen,
          C_{1-10} alkyl unsubstituted or substituted with one to six substituents

independently selected from zero to five halogens and zero or one group selected from
hydroxy and C_{1-3} alkoxy, said alkoxy group being unsubstituted or substituted with

one to three halogens,

20   C_{2-10} alkenyl, unsubstituted or substituted with one to six substituents

independently selected from zero to five halogens and zero or one group selected from
hydroxy and C_{1-3} alkoxy, said alkoxy group being unsubstituted or substituted with

one to three halogens,
          CH_2CO_2H,

25          $CH_2CO_2C_{1-6}$ alkyl,
          CH_2CONHR^a,
          $(CH_2)_{0-2}C_{3-9}$ cycloalkyl,
          $(CH_2)_{0-2}C_{5-12}$ bicycloalkyl,
          $(CH_2)_{0-2}$adamantyl, and
30          $(CH_2)_{0-2}R$;

-111-

wherein said C_{3-9} cycloalkyl and C_{5-12} bicycloalkyl optionally have one to two double bonds, and said C_{3-9} cycloalkyl, C_{5-12} bicycloalkyl, and adamantyl are unsubstituted or substituted with one to six substituents independently selected from (a) zero to five halogens, CH_3, CF_3, OCH_3, and OCF_3, and (b) zero or one phenyl,

5   said phenyl being unsubstituted or substituted with one to four groups independently selected from halogen, OCH_3, OCF_3, CH_3, and CF_3;

R^3 is selected from the group consisting of

hydrogen,

10   C_{1-10} alkyl, unsubstituted or substituted with one to six substituents independently selected from zero to five halogens and zero or one group selected from hydroxy and C_{1-3} alkoxy, said alkoxy group being unsubstituted or substituted with one to three halogens,

          C_{2-10} alkenyl; unsubstituted or substituted with one to six substituents

15   independently selected from zero to five halogens and zero or one group selected from hydroxy and C_{1-3} alkoxy, said alkoxy group being unsubstituted or substituted with one to three halogens,

          YC_{3-9} cycloalkyl,

          YC_{5-12} bicycloalkyl,

20   Yadamantyl, and

          YR;

wherein said C_{3-9} cycloalkyl and C_{5-12} bicycloalkyl optionally have one to two double bonds, and said C_{3-9} cycloalkyl, C_{5-12} bicycloalkyl, and adamantyl are unsubstituted or substituted with one to six substituents independently selected from

25   (a) zero to five halogens, CH_3, CF_3, OCH_3, and OCF_3, and (b) zero or one phenyl, said phenyl being unsubstituted or substituted with one to four groups independently selected from halogen, OCH_3, OCF_3, CH_3, and CF_3;

R is selected from the group consisting of benzodioxolane, furan, tetrahydrofuran,

30   thiophene, tetrahydrothiophene, dihydropyran, tetrahydropyran, pyridine, piperidine, benzofuran, dihydrobenzofuran, benzothiophene, dihydrobenzothiophene, indole, dihydroindole, indene, indane, 1,3-dioxolane, 1,3-dioxane, phenyl, and naphthyl; wherein R is unsubstituted or substituted with one to four groups independently selected from halogen, C_{1-4} alkylthio, C_{1-4} alkylsulfinyl, C_{1-4} alkylsulfonyl, C_{2-4}

alkenylsulfonyl, CN, OH, OCH_3, OCF_3, and C_{1-4} alkyl, said C_{1-4} alkyl being

unsubstituted or substituted with one to five halogens or one substituent selected from

OH and C_{1-3} alkoxy; and

5   Y is selected from $(CH_2)_{0-2}$ and (-HC=CH-);

or, alternatively, R^2 and R^3 taken together form a bridging group R^4, providing a

compound of structural formula Ia:

$$R^1X\text{—}\underset{\underset{\textstyle N\text{—}N}{\overset{\textstyle N}{|}}}{\overset{\textstyle W\text{—}R^4}{\diagdown}}Z$$

(Ia)
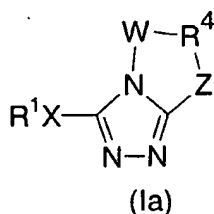
10   wherein R^4 is

a C_{2-8} alkylene group, optionally containing one heteroatom selected from O

and NR^b between two adjacent carbon atoms of said C_{2-8} alkylene group, optionally

containing one to two carbon-carbon double bonds when R^4 is a C_{3-8} alkylene group,

and optionally also comprising a carbon-carbon single bond connecting two non-

15   adjacent carbon atoms of said C_{2-8} alkylene group, or

a C_{4-8} cycloalkyl group,

wherein R^b is selected from the group consisting of hydrogen and C_{1-6} alkyl,

unsubstituted or substituted with one to six substituents independently selected from

zero to five fluorines and zero or one phenyl, said phenyl being unsubstituted or

20   substituted with one to three substituents independently selected from halogen, CH_3,

CF_3, OCH_3, and OCF_3;

wherein R^4 is unsubstituted or substituted with one to five R^c substituents, wherein

each R^c is independently selected from halogen, OH, OCH_3, OCF_3, C_{1-6} alkyl,

C_{2-6} alkenyl, phenyl, biphenyl, C_{3-8} cycloalkyl, C_{1-6} alkyloxycarbonyl, an epoxide

25   group bridging 2 adjacent carbons, and 1,3-dioxolanyl geminally disubstituted onto

one carbon of R^4, wherein each C_{1-6} alkyl and C_{2-6} alkenyl is unsubstituted or

substituted with one to five substituents independently selected from zero to three

halogens and zero to two groups selected from phenyl, C_{1-6} alkyloxycarbonyl, 1,3-

dioxolanyl geminally disubstituted onto one carbon, and CN, and wherein each

phenyl, biphenyl, and C_{3-8} cycloalkyl, either as R^c or as a substituent on R^c, is unsubstituted or substituted with one to three groups independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3;

5   wherein R^4 optionally has a fused phenyl ring, a benzodioxinyl ring, or a dihydrobenzodioxinyl ring, said phenyl ring, benzodioxinyl ring, and dihydrobenzodioxinyl ring being unsubstituted or substituted with one tho three substituents independently selected from halogen, CH_3, CF_3, OCH_3, and OCF_3; and

10   wherein R^4, including said optional fused phenyl ring, benzodioxinyl ring, or dihydrobenzodioxinyl ring and including all substituents on R^4 and said fused phenyl ring, benzodioxinyl ring, and dihydrobenzodioxinyl ring, has no more than 20 carbon atoms.

15   13.   A method for treating, controlling, or delaying hyperglycemia in a mammalian patient in need of such treatment which comprises administering to said patient a therapeutically effective amount of a compound of Claim 12 or a pharmaceutically acceptable salt thereof.

20   14.   A method for treating, controlling, delaying or preventing obesity in a mammalian patient in need of such treatment which comprises administering to said patient a therapeutically effective amount of a compound of Claim 12 or a pharmaceutically acceptable salt thereof.

25   15.   A method for treating, controlling, or delaying insulin resistance in a mammalian patient in need of such treatment which comprises administering to said patient a therapeutically effective amount of a compound of Claim 12 or a pharmaceutically acceptable salt thereof.

30   16.   A method for treating, controlling, or delaying one or more lipid disorders selected from the group consisting of dyslipidemia, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, low HDL, and high LDL in a mammalian patient in need of such treatment which comprises administering to said patient a

therapeutically effective amount of a compound of Claim 12 or a pharmaceutically acceptable salt thereof.

17.   A method for treating, controlling, delaying or preventing atherosclerosis in a mammalian patient in need of such treatment which comprises administering to said patient a therapeutically effective amount of a compound of Claim 12 or a pharmaceutically acceptable salt thereof.

18.   A method for treating, controlling, delaying or preventing in a mammalian patient in need of treatment one or more conditions selected from the group consisting of (1) hyperglycemia, (2) low glucose tolerance, (3) insulin resistance, (4) obesity, (5) lipid disorders, (6) dyslipidemia, (7) hyperlipidemia, (8) hypertriglyceridemia, (9) hypercholesterolemia, (10) low HDL levels, (11) high LDL levels, (12) atherosclerosis and its sequelae, (13) vascular restenosis, (14) pancreatitis, (15) abdominal obesity, (16) neurodegenerative disease, (17) retinopathy, (18) nephropathy, (19) neuropathy, (20) Syndrome X, and other conditions and disorders where insulin resistance is a component or that may be treated by inhibition of the 11β-HSD1 enzyme, wherein the method comprises the administration to said patient of a therapeutically effective amount of a compound of Claim 12 or a pharmaceutically acceptable salt thereof.

19.   A method for treating, controlling, delaying or preventing in a mammalian patient in need of treatment one or more conditions selected from the group consisting of (1) hyperglycemia, (2) low glucose tolerance, (3) insulin resistance, (4) obesity, (5) lipid disorders, (6) dyslipidemia, (7) hyperlipidemia, (8) hypertriglyceridemia, (9) hypercholesterolemia, (10) low HDL levels, (11) high LDL levels, (12) atherosclerosis and its sequelae, (13) vascular restenosis, (14) pancreatitis, (15) abdominal obesity, (16) neurodegenerative disease, (17) retinopathy, (18) nephropathy, (19) neuropathy, (20) Syndrome X, and other conditions and disorders where insulin resistance is a component or that may be treated by inhibition of the 11β-HSD1 enzyme, wherein said treatment comprises the administration to said patient of a therapeutically effective amount of a first compound of Claim 12, or a pharmaceutically acceptable salt thereof, and one or more other compounds selected from the group consisting of:

           (a) DP-IV inhibitors;

(b) insulin sensitizers selected from the group consisting of (i) PPAR agonists and (ii) biguanides;

(c) insulin and insulin mimetics;

(d) sulfonylureas and other insulin secretagogues;

(e) α-glucosidase inhibitors;

(f) glucagon receptor antagonists;

(g) GLP-1, GLP-1 mimetics, and GLP-1 receptor agonists;

(h) GIP,GIP mimetics, and GIP receptor agonists;

(i) PACAP, PACAP mimetics, and PACAP receptor 3 agonists;

(j) cholesterol lowering agents selected from the group consisting of (i) HMG-CoA reductase inhibitors, (ii) sequestrants, (iii) nicotinyl alcohol, nicotinic acid and salts thereof, (iv) PPARα agonists, (v) PPARα/γ dual agonists, (vi) inhibitors of cholesterol absorption, (vii) acyl CoA:cholesterol acyltransferase inhibitors, and (viii) anti-oxidants;

(k) PPARδ agonists;

(l) antiobesity compounds;

(m) ileal bile acid transporter inhibitors;

(n) anti-inflammatory agents, excluding glucocorticoids; and

(o) protein tyrosine phosphatase-1B (PTP-1B) inhibitors.

20. A method for the treatment, control, delay, or prevention of one or more conditions selected from the group consisting of hypercholesterolemia, atherosclerosis, low HDL levels, high LDL levels, hyperlipidemia, hypertriglyceridemia, and dyslipidemia, which method comprises administering to a mammalian patient in need of such treatment a therapeutically effective amount of a compound of Claim 12 and an HMG-CoA reductase inhibitor.

21. The method of Claim 20 wherein the HMG-CoA reductase inhibitor is a statin.

22. The method of Claim 21 wherein the statin is selected from the group consisting of lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, itavastatin, ZD-4522 and rivastatin.

23. A method for the the treatment, control, delay or prevention of atherosclerosis in a mammalian patient in need of such treatment comprising the administration to said patient of an effective amount of a compound of Claim 12, or a pharmaceutically effective salt thereof, and an effective amount of an HMG-CoA reductase inhibitor.

24. The method of Claim 23 wherein the HMG-CoA reductase inhibitor is a statin.

25. A pharmaceutical composition for the treatment, control, delay or prevention of atherosclerosis, comprising a compound of Claim 12, an HMG-CoA reductase inhibitor, and a pharmaceutically acceptable carrier.

26. A pharmaceutical composition comprising
(1) a compound according to Claim 12,
(2) one or more compounds selected from the group consisting of:
     (a) DP-IV inhibitors;
     (b) insulin sensitizers selected from the group consisting of (i) PPAR agonists and (ii) biguanides;
     (c) insulin and insulin mimetics;
     (d) sulfonylureas and other insulin secretagogues;
     (e) α-glucosidase inhibitors;
     (f) glucagon receptor antagonists;
     (g) GLP-1, GLP-1 mimetics, and GLP-1 receptor agonists;
     (h) GIP, GIP mimetics, and GIP receptor agonists;
     (i) PACAP, PACAP mimetics, and PACAP receptor 3 agonists;
     (j) cholesterol lowering agents selected from the group consisting of (i) HMG-CoA reductase inhibitors, (ii) sequestrants, (iii) nicotinyl alcohol, nicotinic acid or a salt thereof, (iv) PPARα agonists, (v) PPARα/γ dual agonists, (vi) inhibitors of cholesterol absorption, (vii) acyl CoA:cholesterol acyltransferase inhibitors, and (viii) anti-oxidants;
     (k) PPARδ agonists;
     (l) antiobesity compounds;
     (m) ileal bile acid transporter inhibitors;
     (n) anti-inflammatory agents other than glucocorticoids; and

(o) protein tyrosine phosphatase-1B (PTP-1B) inhibitors; and
(3) a pharmaceutically acceptable carrier.

27.   The method of Claim 22 further comprising administering a
cholesterol absorption inhibitor.

28.   The method of Claim 27 wherein the cholesterol absorption
inhibitor is ezetimibe.

29.   A method of treating diabetes in a mammal in need thereof
comprising administering to the mammal a therapeutically effective amount of a
compound of Claim 12 in combination with the PPARα/γ dual agonist KRP-297.

Figure 8

Nucleotide sequence of CD26 truncated RANTES chemokine (SEQ ID NO:1):

5'- TATTCCTCGGACACCACACCCTGCTGCTTTGCCTACATTGC-
CCGCCCACTGCCCCGTGCCCACATCAAGGAGTATT
TCTACACCAGTGGCAAGTGCTCCAACCCAGCAGTCGTCTTTGT
CACCCGAAAGAACCGCCAAGTGTGTGCCAACCCAGAGAAGAA
ATGGGTTCGGGAGTACATCAACTCTTTGGAGATGAGC - 3'

Amino acid sequence of CD26 truncated RANTES chemokine (SEQ ID NO:2):

N- YSSDTTPCCFAYIARPLPRAHIKEYFYTSGKCSNPAVVFVTRKN-
RQVCANPEKKWVREYINSLEMS - C

FIGURE 7
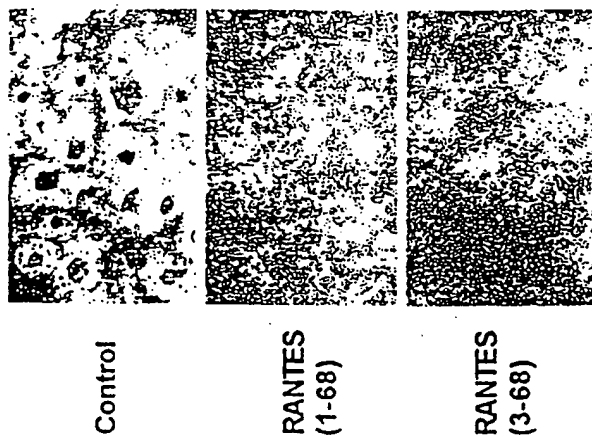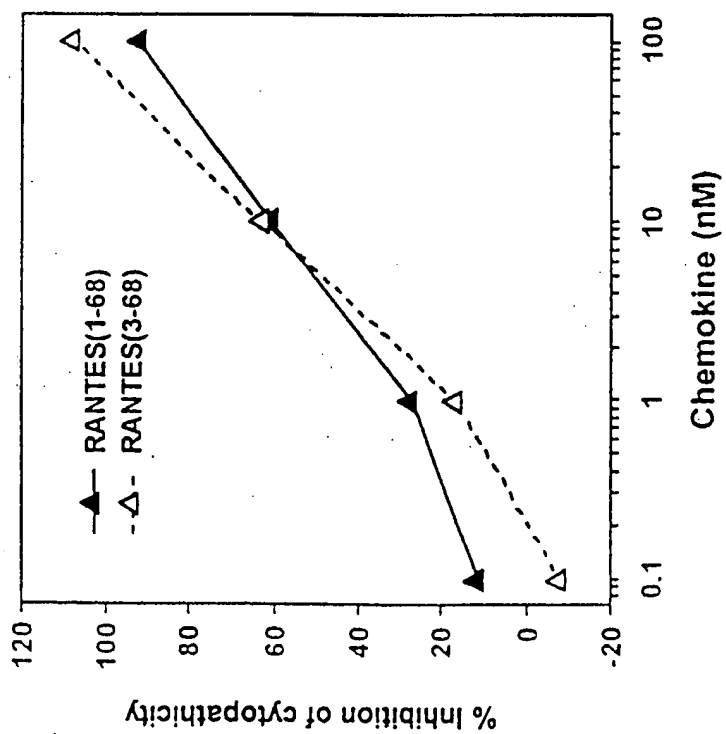
FIGURE 7
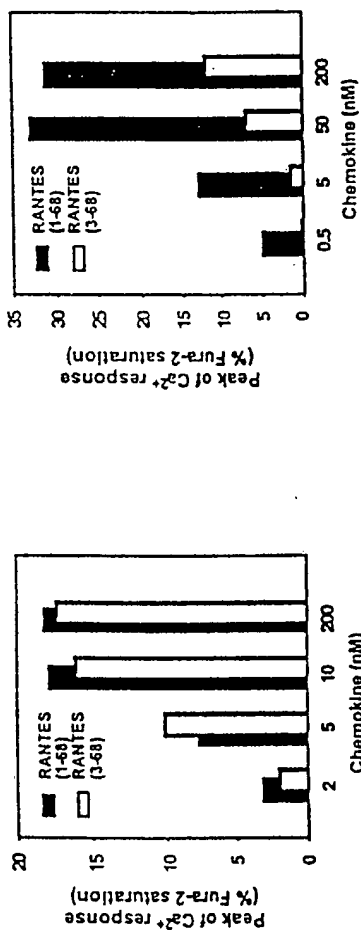
FIGURE 6

FIGURE 6
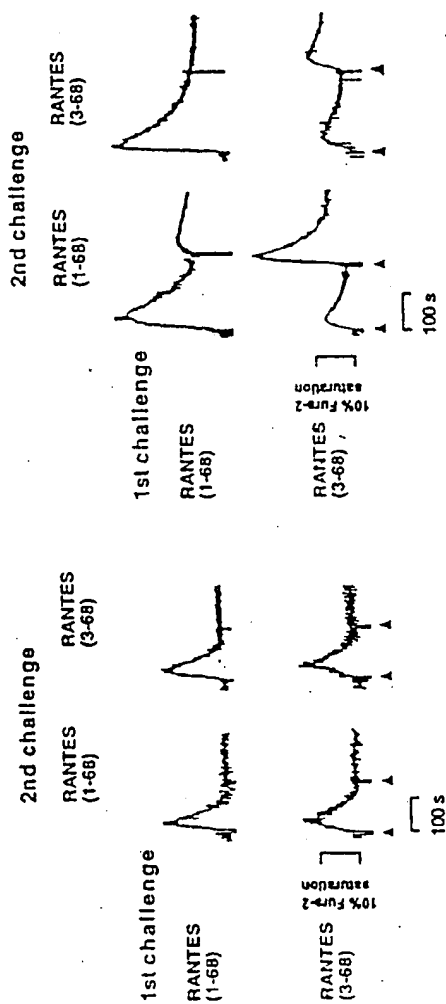
FIGURE 6

FIGURE 6

FIGURE 5
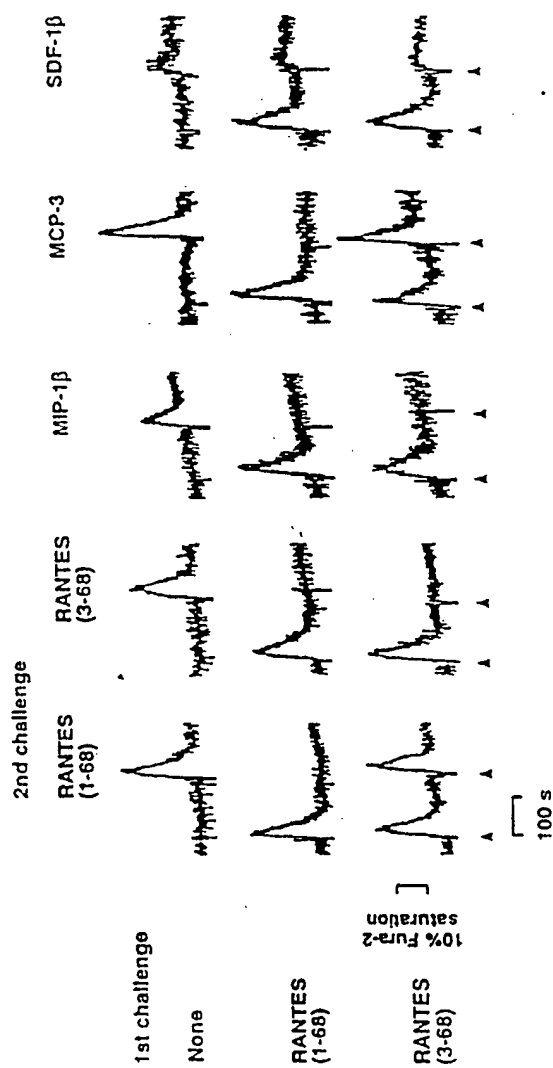
FIGURE 4
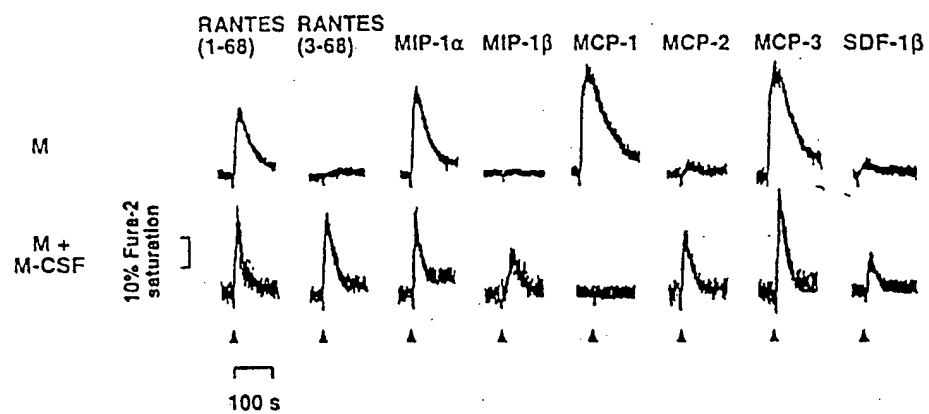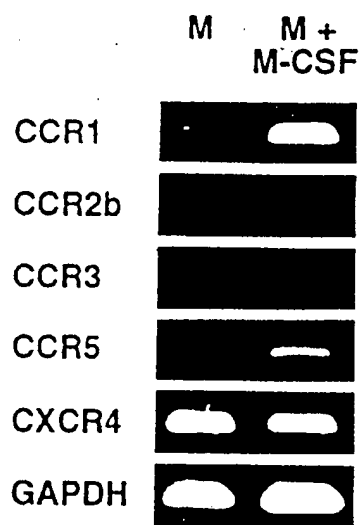
FIGURE 3

2/8

FIGURE 2
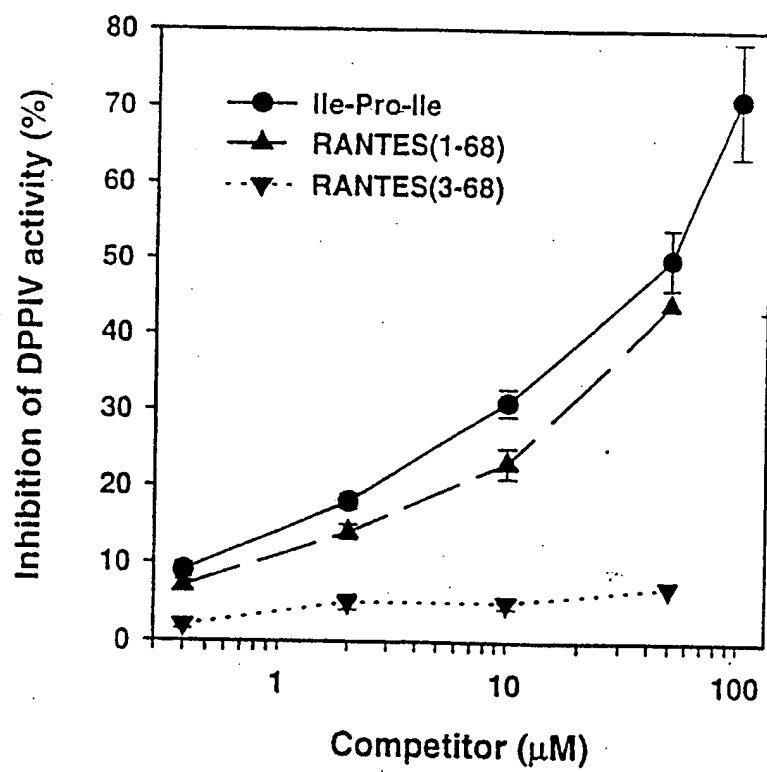
FIGURE 1
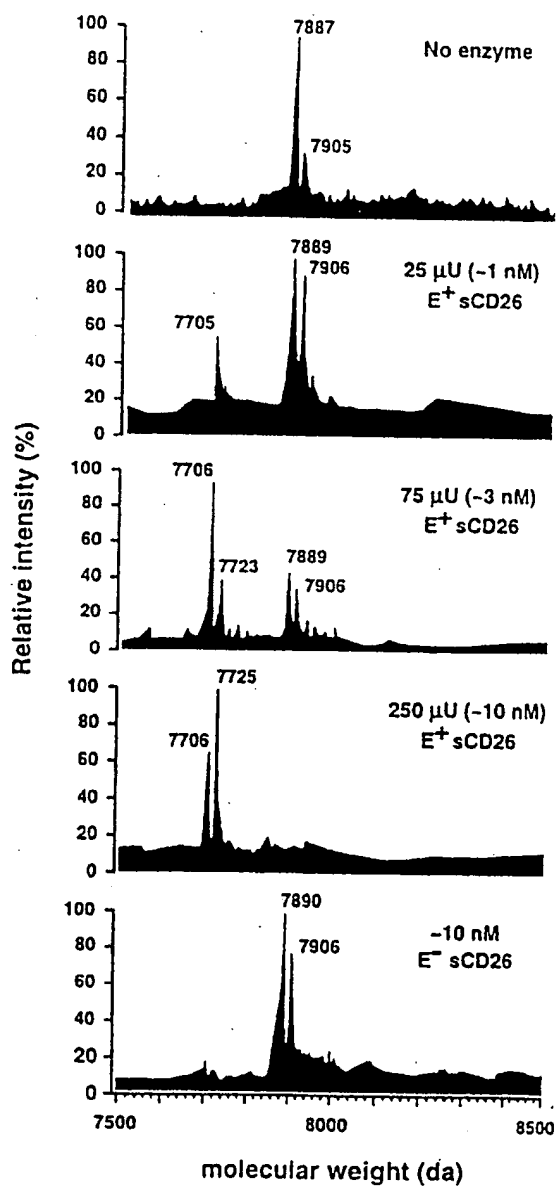
1   46.   Antibodies which bind to DPPIV-truncated chemokine but not to wild-type

2   chemokine.

1   47.   The antibodies as in claims 45 or 46, wherein the chemokine is RANTES.

40.   A method for inhibiting HIV-1 replication in a host cell susceptible to HIV-1 infection, comprising contacting the cell or the host with an effective amount of Dipeptidyl peptidase IV (DPPIV) enzyme such that RANTES is cleaved to produce truncated RANTES, thereby providing antiviral activity and inhibiting HIV-1 replication.

41.   The method as in any of claims 7, 28, 32, 33, 34, 37, or 39, wherein the DPPIV enzyme is CD26.

42.   A method of diagnosis of a subject having a chemokine-associated disorder comprising:

        identifying the presence of a chemokine of interest from a specimen isolated from the subject;

        determining the amino-terminal sequence of the chemokine, wherein a full-length amino acid sequence is indicative of the presence of a wild-type chemokine polypeptide and a truncated amino-terminal sequence is indicative of the presence of a variant chemokine; and

        determining the concentration of wild-type chemokine as compared to variant chemokine, thereby providing a diagnosis of the subject.

43.   The method of claim 42, wherein the determining of the amino-terminal sequence of the chemokine is by contacting the chemokine with an antibody which distinguishes wild-type from variant chemokine polypeptide.

44.   The method of claim 42, wherein the specimen is selected from the group consisting of blood, sputum, urine, saliva, cerebrospinal fluid, and serum.

45.   Antibodies which bind to wild-type chemokine but not to DPPIV-truncated chemokine.

33.   A method for inhibiting Dipeptidyl peptidase IV (DPPIV)-mediated chemokine processing comprising contacting DPPIV with an inhibiting effective amount of a compound which inhibits DPPIV expression or activity.

34.   A method for inhibiting an allergic or inflammatory reaction in a subject, comprising administering to the subject an effective amount of Dipeptidyl peptidase IV (DPPIV) enzyme such that a chemokine is cleaved to produce a truncated chemokine, thereby inhibiting an allergic or inflammatory reaction.

35.   The method of claim 34, wherein the chemokine is eotaxin.

36.   The method of claim 34, wherein the subject is a human.

37.   A method for accelerating angiogenesis or wound healing in a subject, comprising administering to the subject an effective amount of an inhibitor of Dipeptidyl peptidase IV (DPPIV) enzyme activity or gene expression or a DPPIV-insensitive chemokine, such that chemokine processing is inhibited, thereby accelerating angiogenesis or wound healing.

38.   The method of claim 37, wherein the chemokine is IP-10.

39.   The method of claim 37, wherein the DPPIV-insensitive chemokine is a wild-type chemokine with the proviso that alanine or proline at position 2 is replaced with any amino acid other than alanine or proline.

1   25.   The method of claim 21, wherein the administering is *in vivo*.

1   26.   A pharmaceutical composition comprising the polypeptide of SEQ ID NO:2

2   in a pharmaceutically acceptable carrier.

1   27.   A pharmaceutical composition comprising CD26 polypeptide in a pharmaceu-

2   tically acceptable carrier.

1   28.   A method for producing a variant chemokine having an activity different from

2   the activity of the wild-type chemokine, comprising contacting the wild-type

3   chemokine with an N-terminal processing effective amount of Dipeptidyl

4   peptidase IV (DPPIV), thereby truncating the chemokine and producing a

5   variant chemokine.

1   29.   The method of claim 28, wherein the chemokine contains a proline or an

2   alanine at position 2 from the N-terminus.

1   30.   The method of claim 29, wherein the chemokine is selected from the group

2   consisting of RANTES, MIP-1, IP-10, eotaxin, MDC and MCP-2.

1   31.   The method of claim 29, wherein the chemokine is RANTES.

1   32.   A method for inhibiting HIV-1 replication in a host cell susceptible to HIV-1

2   infection, comprising contacting the cell or the host with an effective amount

3   of Dipeptidyl peptidase IV (DPPIV) enzyme such that macrophage-derived

4   chemokine (MDC) is cleaved to produce truncated MDC, thereby providing

5   antiviral activity and inhibiting HIV-1 replication.

1   15.   The method of claim 14, wherein the contacting is by *in vivo* administration to a
2   subject.

1   16.   The method of claim 14, wherein the polypeptide is administered by intravenous,
2   intramuscular or subcutaneous injection.

1   17.   The method of claim 14, wherein the polypeptide is formulated in a pharmaceuti-
2   cally acceptable carrier.

1   18.   A method of treating a subject having or at risk of having an HIV infection or
2   disorder, comprising administering to the subject, a therapeutically effective amount
3   of a polypeptide of SEQ ID NO:2, wherein the polypeptide inhibits cell-cell fusion
4   in cells infected with HIV.

1   19.   The method of claim 18, wherein the subject is suffering from AIDS or ARC.

1   20.   The method of claim 18, wherein the polypeptide is formulated in a pharmaceutically
2   acceptable carrier.

1   21.   A method of treating a subject having an HIV-related disorder associated with
2   expression of CCR5 comprising administering to an HIV infected or susceptible cell
3   of the subject, a polypeptide of SEQ ID NO:2 or a nucleic acid sequence encoding
4   the polypeptide of SEQ ID NO:2 or other variant chemokine.

1   22.   The method of claim 21, wherein the polypeptide or nucleic acid is introduced into
2   the cell using a carrier.

1   23.   The method of claim 22, wherein the carrier is a vector.

1   24.   The method of claim 21, wherein the administering is *ex vivo*.

1.   7.   A method for identifying a compound which modulates Dipeptidyl peptidase IV
2   (DPPIV)-mediated chemokine processing comprising:
3   a)   incubating components comprising the compound, DPPIV and a
4   chemokine under conditions sufficient to allow the components to
5   interact; and
6   b)   determining the N-terminal amino acid sequence of the chemokine
7   before and after incubating in the presence of the compound.

1   8.   The method of claim 7, wherein the modulating is inhibition of DPPIV-mediated
2   chemokine processing.

1   9.   The method of claim 7, whererin the modulating is stimulation of DPPIV-mediated
2   chemokine processing.

1   10.   The method of claim 7, wherein the compound is a peptide.

1   11.   The method of claim 7, wherein the compound is a peptidomimetic.

1   12.   The method of claim 7, wherein the DPPIV is expressed in a cell.

1   13.   The method of claim 7, wherein the chemokine contains a proline or an alanine at
2   position 2 from the N-terminus.

1   14.   A method of inhibiting membrane fusion between HIV and a target cell or between
2   an HIV-infected cell and a CD4 positive uninfected cell comprising contacting the
3   target or CD4 positive cell with a fusion-inhibiting effective amount of the
4   polypeptide of SEQ ID NO:2.

What Is Claimed Is:

1   1.   A substantially pure polypeptide having an amino acid sequence as set forth in SEQ

2   ID NO:2.

1   2.   An isolated polynucleotide which encodes an amino acid sequence as set forth in

2   SEQ ID NO:2.

1   3.   An isolated polynucleotide selected from the group consisting of:

2   a)   SEQ ID NO:1;

3   b)   SEQ ID NO:1,wherein T can also be U;

4   c)   nucleic sequences complementary to SEQ ID NO:1;

5   d)   fragments of a), b), or c) that are at least 15 bases in length and that

6   will hybridize to DNA which encodes SEQ ID NO:2.

1   4.   An expression vector containing in operable linkage the polynucleotide as in claim

2   2.

1   5.   A host cell containing the vector of claim 4.

1   6.   The host cell of claim 5, wherein the cell is a eukaryotic cell.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, that the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

protein-1beta-mediated signaling are independent functions of the chemokine receptor CCR5. J. Biol. Chem. 272:6854-6857.

29.Mallet, A.I., and I. Kay. 1995. Characterization of chemokine proinflammatory proteins by combined liquid chromatography-mass spectrometry. Biochem. Soc. Trans. 23:911-913.

30.Noso, N.M., Sticherling, J. Bartels, A.I. Mallet, E. Christophers, and J.-M. Schr÷der. 1996. Identification of an N-terminally truncated form of the chemokine RANTES and granulocyte-macrophage colony-stimulating factor as major eosinophil attractants released by cytokine-stimulated dermal fibroblasts. J. Immunol. 156:1946-1953.

31.Tanaka, T., J.S. Duke-Cohan, J. Kameoka, A. Yaron, I. Lee, F.F. Schlossman, and C. Morimoto. 1994. Enhancement of antigen-induced T-cell proliferation by soluble CD26/dipeptidyl peptidase IV. Proc. Natl. Acad. Sci. U.S.A. 91:3082-3086.

32.Duke-Cohan, J.S., C. Morimoto, J.A. Rocker, and S. Schlossman. 1996. Serum high molecular weight dipeptidyl peptidase IV (CD26) is similar to a novel antigen DPPT-L released from activated T cells. J. Immunol. 156:1714-1721.

33.Feng, Y., C.C. Broder, P.E. Kennedy, and E.A. Berger. 1996. HIV-1 entry cofactor: Functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science. 272:872-876.

34.Berson, J.F., D. Long, B. J. Doranz, J. Rucker, F. R. Jirik, and R. W. Doms. 1996. A seven-transmembrane domain receptor involved in fusion and entry of T-cell-tropic human immunodeficiency virus type 1 strains. J. Virol. 70:6288-6295.

35.Cheng-Mayer, C., M. Quiroga, J.W. Tung, D. Dina, and J.A. Levy. 1990. Viral determinants of human immunodeficiency virus type 1 T-cell or macrophage tropism, cytopathogenicity, and CD4 antigen modulation. J. Virol. 64:4390-4398.

36.Wang, J.M., D.W. McVicar, J.J. Oppenheim, and D.J. Kelvin. 1993. Identification of RANTES receptors on human monocytic cells: competition for binding and desenzitization by homologous chemotactic cytokines. J. Exp. Med. 177:699-705.

20. Oberlin, E., A. Amara, F. Bachelerie, C. Bessia, J.-L. Virelizier, F. Arenzana-Seisdedos, O. Schwartz, J.-M. Heard, I. Clark-Lewis, D. F. Legler, M. Loetscher, M. Baggiolini, and B. Moser. 1996. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. Nature. 382:833-835.

21. Van Damme, J., P. Proost, J.-P. Lenaerts, and G. Opdenakker. 1992. Structural and functional identification of two human, tumor-derived monocyte chemotactic proteins (MCP-2 and MCP-3) belonging to the chemokine family. J. Exp. Med. 176:59-65.

22. Alkathib, G., C. Combadiere, C.C. Broder, Y. Feng, P.E. Kennedy, P.M. Murphy, and E.A. Berger. 1996. CC CKR5: A RANTES, MIP-1(, MIP-1(, receptor as a fusion cofactor for macrophage-tropic HIV-1. Science. 272:1955-1958.

23. Choe, H., M. Farzan, Y. Sun, N. Sullivan, B. Rollins, P.D. Ponath, L. Wu, C.R. Mackay, G. LaRosa, W. Newman, N. Gerard, C. Gerard, and J. Sodroski. 1996. The (-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell. 85:1135-1148.

24. Doranz, B.J., J. Rucker, Y. Yi, R.J. Smyth, M. Samson, S.C. Peiper, M. Parmentier, R.G. Collman, and R.W. Doms. 1996. A dual-tropic primary HIV-1 isolate that uses fusin and the (-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell. 85:1149-1158.

25. Deng, H., R. Liu, W. Ellmeier, S. Choe, D. Unutmaz, M. Burkhart, P. Di Marzio, S. Marmon, R.E. Sutton, C.M. Hill, C.B. Davis, S.C. Peiper, T.J. Schall, D.R. Littman, and N.R. Landau. 1996. Identification of a major co-receptor for primary isolates of HIV-1. Nature. 381:661-673.

26. Dragic, T., V. Litwin, G.P. Allaway, S.R. Martin, Y. Huang, K.A. Nagashima, C. Cayanan, P.J. Maddon, R.A. Koup, J.P. Moore, and W.A. Paxton. 1996. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. Nature 381:667-673.

27. Oravecz, T., M. Pall, and M.A. Norcross. 1996. (-chemokine inhibition of monocytotropic HIV-1 infection: Interference with a postbinding fusion step. J. Immunol. 157:1329-1332.

28. Farzan, M., H. Choe, K.A. Martin, Y. Sun, M. Sidelko, C.R. Mackay, N.P. Gerard, J. Sodroski, and C. Gerard. 1997. HIV-1 entry and macrophage inflammatory

11. Oravecz, T., G. Roderiquez, J. Koffi, J. Wang, M. Ditto, D. C. Bou-Habib, P. Lusso, and M. A. Norcross. 1995. CD26 expression correlates with entry, replication and cytopathicity of monocytotropic HIV-1 strains in a T-cell line. Nature Med. 1:919-926.

12. Samson, M., O. Labbe, C. Mollereau, G. Vassart, and M. Parmentier. 1996. Molecular cloning and functional expression of a new human CC-chemokine receptor gene. Biochemistry. 35:3362-3367.

13. Tanaka, T., D. Camerini, B. Seed, Y. Torimoto, N.H. Dang, J. Kameoka, H.N. Dahlberg, S.F. Schlossman, and C. Morimoto. 1992. Cloning and functional expression of the T cell activation antigen CD26. J Immunol. 149:481-486.

14. Davis, S.J., H.A. Ward, M.J. Puklavec, A.C. Willis, A.F. Williams, and A.N. Barclay. 1990. High level expression in Chinese hamster ovary cells of soluble forms of CD4 T lymphocyte glycoprotein including glycosylation variants. J. Biol. Chem. 265:10410-10418.

15. McCaughan, G.W., J.E. Wickson, P.F. Creswick, and M.D. Gorrell. 1990. Identification of the bile canalicular cell surface molecule GP110 as the ectopeptidase dipeptidyl peptidase IV: an analysis by tissue distribution, purification and N-terminal amino acid sequence. Hepatology. 11:534-544.

16. Schall, T.J., J. Jongstra, B.J. Dyer, J. Jorgensen, C. Clayberger, M.M. Davis, and A.M. Krensky. 1988. A human T cell-specific molecule is a member of a new gene family. J. Immunol. 141:1018-1025.

17. Rahfeld, J., M. Schierhorn, B. Hartrodt, K. Neubert, and J. Heins. 1991. Are diprotin A (Ile-Pro-Ile) and diprotin B (Val-Pro-Leu) inhibitors or substrates of dipeptidyl peptidase IV? Biochim. Biophys. Acta. 1076:314-316.

18. Nagasawa, T., H. Kikutani, and T. Kishimoto. 1994. Molecular cloning and structure of a pre-B-cell growth-stimulating factor. Proc. Natl. Acad. Sci. U.S.A. 91:2305-2309.

19. Bleul, C.C., M. Farzan, H. Choe, C. Parolin, I. Clark-Lewis, J. Sodroski, and T.A. Springer. 1996. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. Nature. 382:829-833.

References

1.Murphy, P.M. 1996. Chemokine receptors: structure, function and role in microbial pathogenesis. Cytokine Growth Factor Rev. 7:47-64.

2.Sica, A., A. Saccani, A. Borsatti, C.A. Power, T.N. Wells, W. Luini, N. Polentarutti, S. Sozzani, and A. Mantovani. 1997. Bacterial lipopolysaccharide rapidly inhibits expression of C-C chemokine receptors in human monocytes. J. Exp. Med. 185:969-974

3.Weber, M., M. Uguccioni, M. Baggiolini, I. Clark-Lewis, and C.A. Dahinden. 1996. Deletion of the NH2-terminal residue converts monocyte chemotactic protein 1 from an activator of basophil mediator release to an eosinophil chemoattractant. J. Exp. Med. 183:681-685.

4.Gong, J.-H., M. Uguccioni, B. Dewald, M. Baggiolini, and I. Clark-Lewis. 1996. RANTES and MCP-3 antagonists bind multiple chemokine receptors. J. Biol. Chem. 271:10521-10527.

5.Arenzana-Seisdedos, F., J.-L. Virelizier, D. Rousset, I. Clark-Lewis, P. Loetscher, B. Moser, and M. Baggiolini. HIV blocked by chemokine antagonist. 1996. Nature. 383:400.

6.Murphy, P.M. 1994. The molecular biology of leukocyte chemoattractant receptors. Annu. Rev. Immunol. 12:593-633.

7.Walter, R., W.H. Simmons, and T. Yoshimoto. 1980. Proline specific endo- and exopeptidases. Mol. Cell. Biochem. 30:111-127.

8.Fox, D.A., R.E. Hussey, K.A. Fitzgerald, O. Acuto, C. Poole, L. Palley, J.F. Daley, S.F. Schlossman, and E.L. Reinherz. 1984. Ta1, a novel 105 kD human T cell activation antigen defined by a monoclonal antibody. J. Immunol. 133:1250-1256.

9.Hegen, M., G. Niedobitek, C.E. Klein, H. Stein, and B. Fleischer. 1990. The T cell triggering molecule Tp103 is associated with dipeptidyl aminopeptidase IV activity. J. Immunol., 144:2908-2914.

10.Fleischer, B. 1994. CD26: a surface protease involved in T-cell activation. Immunol. Today. 15:180-184.

Table 1.   Chemokine cleavage products after digestion with sCD26.

Chemokine	NH2-terminal dipeptide	CD26 cleavage	Molecular masses by mass spectrometry (Da)			
			Full length		Truncated	
			Theoretical	Observed	Theoretical	Observed
Eotaxin	GP	Yes	8361	8361	8207	8207
IP-10	VP	Yes	8633	8637 / 8751*	8437	8440 / 8555*
MCP-1	QP	No	8681	8678	8456	ND ‖
MCP-2	QP	Yes	8910	8909	8685	8686 / 8703#

* Tentatively identified as [M + trifluoroacetic acid (TFA)]+; molecular mass of TFA is 114 Da.

Tentatively identified as [M + H2O]+.

‖ ND = not detected.

susceptible to infection by M-tropic variants of HIV-1. We have shown that macrophages also express CXCR4, the coreceptor for T cell line-tropic HIV-1 variants (33-34), as assessed by receptor transcript abundance and functional activity of the CXCR4 ligand SDF-1(. Nevertheless, activated macrophages are relatively resistant to infection by T cell line-tropic HIV-1 variants (35), which suggests that factors other than CXCR4 may also be required for efficient infection of macrophages by these types of viruses.

Removal of two NH2-terminal residues by CD26 abolishes the interaction of RANTES with CCR1, but does not affect the anti-HIV-1 activity or the CCR5 signaling properties of the chemokine. Proline residues also influence the susceptibility of proximal peptide bonds to proteolytic enzymes (6), and so the removal of such residues by CD26 may also reduce the half-life of RANTES and other chemokines during an inflammatory response. It will be important to determine whether CD26-mediated cleavage is a general mechanism for changing the receptor specificity and functional activity of other chemokines, including those examined in this study (MCP-2, eotaxin, and IP-10).

Many, but not all CC- and CXC-chemokines contain X-Pro- or X-Ala- amino-terminal sequence and are potential substrates of DPPIV. We are currently exploring whether the inability of CD26 to cleave MCP-1 is due to aggregation of this chemokine under these experimental conditions or to a conformational requirement of the enzyme that is not fulfilled by MCP-1. Selectivity of CD26 activity on chemokines may function to reduce redundancy in chemokine target cell specificity as illustrated by the different activity of full-length and truncated RANTES on monocytes versus macrophages. Finally, truncated analogs of chemokines with selective activity on distinct functional receptors, or analogs that resist CD26 cleavage, may prove therapeutically beneficial in blocking or inducing the infiltration of specific subsets of effector cells mediating inflammation, allergy and anti-tumor responses.

activity through CCR5, competitive inhibition of HIV-1 infection does not require the NH2-terminal Ser-Pro residues of RANTES.

The CD26 cleavage product of RANTES, RANTES(3-68), acts as a chemokine agonist with altered receptor-specificity. Hydrolysis by CD26 might explain why RANTES(3-68) has been isolated as a second component in addition to intact RANTES from culture supernatants of stimulated human fibroblasts, skin samples, and platelet preparations (29, 30). The CC-chemokines RANTES, MCP-2, and eotaxin, and the CXC-chemokine IP-10 are the first immune modulators and the longest polypeptides identified as natural substrates for CD26.

CD26 exists in both soluble and membrane-expressed forms. Secreted forms of CD26 have been identified in cell cultures and in human serum (31, 32), although CD26 may be more active when expressed as an ectoenzyme at high concentrations on endothelial cells, hepatocytes, kidney brush border membranes, and leukocytes (10). Up-regulation of CD26 expression on T lymphocytes and macrophages has been linked to cell activation and development of immunological memory (10). Thus, activation-induced changes in CD26 expression could affect the course of an inflammatory response by modifying the target cell specificity of RANTES or other chemokines, and by regulating the equilibrium between the migrating cell subsets. We are currently addressing whether cells with different levels of CD26 expression (e.g. naive versus memory T cells) secrete truncated forms of RANTES or other chemoattractants, or are capable of modifying exogenous chemokines.

The differential effects of CD26-truncated RANTES on monocytes versus macrophages illustrate a role for cell differentiation in regulating chemokine sensitivity through altered receptor expression. Our functional and receptor transcript data indicate that CCR1 and CCR2b may be the two principal CC chemokine receptors in resting monocytes, although other unidentified and functionally overlapping receptors may also contribute to chemokine function. Cell differentiation markedly changes the pattern of chemokine sensitivity by reducing CCR2b expression, thereby rendering the cells resistant to MCP-1, while increasing CCR5 expression, thereby augmenting the responses to CD26-truncated RANTES and MIP-1(. An increase in CCR5 expression also may render macrophages more

EXAMPLE 6

CCR1- and CCR5-mediated signaling of RANTES

HEK-293 cells expressing CCR5 and HOS-CD4 cells expressing CCR1 were loaded with Fura-2 and exposed to various concentrations of RANTES(1-68) or RANTES(3-68). The two RANTES variants showed similar abilities to increase $[Ca2+]i$ in the CCR5 transfectant (Fig. 6 A); the responses were dose dependent, with 10 nM of each variant sufficient to induce a maximal Ca2+ response. In contrast, in the cells expressing CCR1, the amount of RANTES(3-68) required to produce a detectable Ca2+ response was ~100 times that for RANTES (1-68) (Fig. 6 B); the effect of RANTES(1-68) saturated at 50 nM, whereas that of RANTES(3-68) appeared not to have achieved saturation at 200 nM. Furthermore, bidirectional cross-desensitization between the two RANTES variants was evident only with the cells expressing CCR5 (Fig. 6 C); in the CCR1 transfectant, cross-desensitization was induced by full-length RANTES but not by the truncated form, which also did not exhibit self-desensitization (Fig. 6 D). Control cells transfected with vector alone or with vectors encoding CCR2b, CCR3, or CXCR4 did not respond to these ligands (data not shown). These results thus confirm that the native and CD26-truncated RANTES variants exhibit markedly different activities at the CCR1 receptor.

EXAMPLE 7

RANTES(3-68) is a potent inhibitor of HIV-1

In addition to their function in chemotaxis, RANTES, MIP-1(, and MIP-1(each inhibit HIV-1 infection by competitive binding to CCR5 (22-27), and this inhibition does not require receptor-mediated cell signaling (27, 28). To examine whether removal of the two NH2-terminal residues affects the antiviral activity of RANTES, we mixed HOS-CD4 cells expressing recombinant CCR5 and PM1 cells chronically infected with the M-tropic recombinant MV3-HXB2 virus and cocultured them in the absence or presence of various concentrations of RANTES(1-68) or RANTES (3-68). Both RANTES variants inhibited HIV-1-induced syncytium formation and cytopathicity (Fig. 7). Thus, similar to signaling

EXAMPLE 5

RANTES(3-68) is a chemokine agonist, with altered receptor specificity

Agonists that act at common chemokine receptors block each other's activity as a result of receptor desensitization, whereas responses to chemokines that act at different
5   receptors are generally not affected (1, 6). We therefore performed comparative desensitization experiments to define the types of receptors that mediate the effects of native versus truncated RANTES in macrophages (Fig. 5). Macrophages that were stimulated first with 100 nM RANTES(1-68) did not exhibit a second Ca2+ response when challenged with the same dose of either full-length or truncated RANTES. In contrast, cells stimulated with 100 nM
10 RANTES(3-68) fully retained their ability to respond to a subsequent challenge with full-length RANTES, but were desensitized to the effect of the truncated form. These results suggest that the receptor repertoire available for truncated RANTES is more restricted than that available for the native chemokine.   To characterize further the receptor usage of the different forms of RANTES and other chemokines, we also studied the sensitivity of
15 MIP-1(-, MCP-3-, and SDF-1(-induced Ca2+ responses to RANTES-mediated receptor desensitization (Fig. 5). Of the known receptors, RANTES signals via CCR1, CCR4, and CCR5, whereas MIP-1(acts at CCR5 exclusively and MCP-3 binds only to CCR1 and CCR2b at the concentrations used in our experiments (1, 6). The only receptor known to bind SDF-1(is CXCR4 (19, 20). Pretreatment of macrophages with full-length RANTES
20 blocked the ability of MIP-1(and MCP-3, but not that of SDF-1(, to increase [Ca2+]i. In contrast, RANTES(3-68) desensitized cells to the effect of MIP-1(but did not affect the response to MCP-3 or SDF-1. These results are consistent with previous data on RANTES-induced receptor desensitization (1) and with our data on chemokine receptor mRNA abundance (Fig. 3). They suggest that, in M-CSF-activated macrophages, full-length
25 RANTES shares CCR1 and CCR5 receptors with MCP-3 and MIP-1(, respectively. Our results also indicate that, without its two NH2-terminal residues, RANTES is still able to signal via CCR5 but can no longer act at the CCR1 receptor.

EXAMPLE 4

CD26-specific truncation of RANTES modifies its target cell specificity

To investigate the functional significance of DPPIV-mediated truncation of RANTES, we compared the effects of chemically synthesized RANTES(1-68) and RANTES(3-68) on monocytes and monocyte-derived macrophages. Both resting cells and cells activated with M-CSF were analyzed because RT-PCR revealed marked changes in the abundance of chemokine receptor transcripts in response to M-CSF activation (Fig. 3). In resting cells, transcripts encoding the chemokine receptors CCR1, CCR2b, or CXCR4, as well as control glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA, were readily detectable, whereas CCR5 receptor transcripts were virtually absent. After differentiation to macrophages, the intensity of the CXCR4 and GAPDH signals remained virtually unchanged, whereas the abundance of CCR1 and CCR5 mRNAs increased substantially and the CCR2b transcript virtually disappeared. CCR3 mRNA was not detected in either cell type.

Transient changes in the cytosolic free Ca2+ concentration ([Ca2+]i) were recorded after stimulation of monocytes or macrophages with an optimal concentration of RANTES-(1-68) or RANTES(3-68), and the effects were compared with those of other chemokines (Fig. 4). Addition of 100 nM RANTES(1-68) to cells loaded with the fluorescent Ca2+ probe Fura-2 induced a rapid increase in [Ca2+]i in both monocytes and macrophages. In contrast, the same concentration of RANTES(3-68) increased [Ca2+]i in macrophages but not in monocytes. Among the other chemokines tested, macrophage inflammatory protein-1((MIP-1(), monocyte chemotactic protein-1 (MCP-1), MCP-3 (1, 6), and stromal-derived factor-1((SDF-1() (18-20) also increased [Ca2+]i in resting monocytes, whereas MCP-2 (21) induced a barely detectable response and MIP-1((1, 6) was inactive. On the basis of the previously described receptor specificities of these chemokines (1, 6, 19, 20), the obtained activity pattern is consistent with expression of CCR1, CCR2b, and CXCR4 receptors on monocytes (Fig. 3). Macrophages showed marked Ca2+ responses to MIP-1(, MIP-1(, MCP-2, MCP-3, and SDF-1(, but were resistant to MCP-1, consistent with the presence of transcripts encoding CCR1, CCR5, and CXCR4, and the absence of those encoding CCR2b, in these cells (Fig. 3).

EXAMPLE 2

RANTES, MCP-2, eotaxin, and IP-10 are substrates of CD26

ES-MS analysis revealed that 100 nM rhRANTES underwent partial to complete
hydrolysis when incubated overnight at 37oC with increasing amounts (25 to 250 (U) of
sCD26 (Fig. 1). Taking into account cationization (K+) of the multiply charged ions, the
measured molecular masses of the native and degraded polypeptides corresponded to the
theoretical masses of full-length (residues 1 to 68) and truncated (residues 3 to 68) forms of
RANTES, respectively. The calculated difference between the molecular masses of the native
and the truncated forms ranged from 183 to 185 daltons, which is consistent with the
expected mass (184 daltons) of a released Ser-Pro dipeptide, the predicted NH2-terminus of
RANTES (16). In contrast to the effect of enzymatically active sCD26, shortened RANTES
was not generated by incubation of the chemokine with a mutant sCD26 deficient in enzyme
activity (Fig. 1). RANTES also inhibited, possibly in a competitive manner, the rapid
hydrolysis of a pNA-conjugated Gly-Pro dipeptide by human placental DPPIV, as measured
in a colorimetric enzyme assay (Fig. 2). The efficacy of inhibition by chemically synthesized
RANTES(1-68) was similar to that observed with the DPPIV substrate and competitive
inhibitor Ile-Pro-Ile (Diprotin A) (17), whereas RANTES (3-68) did not inhibit the reaction.

EXAMPLE 3

Sensitivity to CD26-mediated cleavage

Sensitivity to CD26-mediated cleavage was not a unique property of RANTES (Table
1.). Cleavage products with the predicted molecular masses were also evident in samples of
MCP-2, eotaxin and IP-10 after incubation with sCD26. In contrast, MCP-1, which has a
62% sequence similarity with MCP-2 including the NH2-terminal QP dipeptides, was not
cleaved by the enzyme under the same experimental conditions.

<u>Cytosolic calcium measurements.</u>

Cells (107/ml) were washed and incubated in the dark at 37oC for 45 min in Ca2+
buffer [136 mM NaCl, 4.8 mM KCl, 5 mM glucose, 1 mM CaCl2, 20 mM Hepes (pH 7.4)]
supplemented with 5 (M Fura-2 acetoxymethyl ester that had been premixed with 10%
Pluronic« F-127 (Molecular Probes). The cells were then washed and resuspended at 2 (106
cells/ml in Ca2+ buffer containing BSA (1 mg/ml), and portions (2 ml) of the cell suspension
were exposed at different time points in a stirred cuvette at 37oC to chemokines.
Fluorescence was monitored with a Photon Technology International d scan (South
Brunswick, NJ), and data were recorded as the relative ratio of fluorescence at excitation
wavelengths of 340 and 380 nm, with emission measured at 510 nm. After each measure-
ment, maximal and minimal fluorescence were assessed by addition of 20 (M ionomycin
followed by 5 mM MnCl2.

Assay for HIV-1-induced cytopathicity. HOS-CD4.CCR5 cells (2 (104) were incubated for
1 hour at 37oC with RANTES variants in 150 (l of culture medium containing 20% FCS,
and were then mixed with 50 (l (2 (105 cells/ml) of uninfected PM1 cells or PM1 cells
chronically infected with MV3-HXB2 virus. After 3 days, photomicrographs of cultures were
taken and cell viability was measured by adding of 50 (l of 1 mg/ml
2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide solution containing
20 (M phenazine methosulfate and recording the OD at 450 nm. Data are expressed as the
percentage inhibition of cytopathicity [calculated as 100% ((R (V)/(U (V), where U, V,
and R represent OD values obtained for HOS-CD4.CCR5 cells cultured with uninfected PM1
cells, or with HIV-1-infected cells in the absence or presence of chemokine, respectively].

ES-MS analysis of samples was performed in 50% acetonitrile, supplemented with 0.1% (v/v) glacial acetic acid, using a Finnigan (San Jose, CA) TSQ 7000 triple-stage quadrupole mass spectrometer. Several scans were summed to obtain the final spectrum.

Peptide synthesis. Full-length and truncated RANTES were synthesized with an Applied Biosystems (Foster City, CA) peptide synthesizer according to fluorenyl methoxycarbonyl (FMOC) chemistry. FMOC-protected amino acids were added stepwise with ninhydrin monitoring at each cycle. The peptides were folded by air oxidation and purified by RP HPLC. Peptide sequences were confirmed by amino acid analysis and Edman sequence analysis, and the molecular masses were confirmed by ES-MS analysis. There was no substantial difference in the activities of chemically synthesized full-length RANTES and rhRANTES(1-68) as judged by the Ca2+ influx and anti-HIV-1 assays used in this study.

Colorimetric DPPIV enzyme assay.

The p-nitroanilide (pNA)-conjugated Gly-Pro dipeptide substrate and test competitors were mixed and added to human placental DPPIV (Enzyme System Products), and the resulting mixture was incubated at room temperature in a final volume of 150 (l containing 50 mM tris-HCl (pH 8.0) and 0.15 M NaCl. The final concentrations of DPPIV and Gly-Pro-pNA were 1.25 mU/ml and 400 (M, respectively. The kinetics of the enzyme reaction were monitored by measuring absorbance at 405 nm with a Vmax kinetic microplate reader (Molecular Devices, Menlo Park CA). The percentage inhibition of enzyme activity was calculated from the maximal velocity for each sample and from that apparent in the absence of competitor (100% activity).

RT-PCR analysis. Isolated total cellular RNA of monocytes was subjected to first-strand cDNA synthesis. PCR amplification of cDNA was performed for 30 cycles (92 oC for 1 min, 40oC for 1 min, 72oC for 1 min) with primers specific for CCR1, CCR2b, CCR3, CCR5, CXCR4, and GAPDH. Separated products were stained with SYBR Green I (Molecular Probes, Eugene, OR).

EXAMPLE 1

Materials and Methods

Cell cultures and transfections. Monocytes were isolated from human PBMCs of healthy donors by counter-current centrifugal elutriation. Monocyte-derived macrophages were prepared by culturing monocytes for 6 days at a density of 106 cells/ml in serum-free macrophage medium (Gibco BRL, Grand Island, NY) supplemented with recombinant human (rh) M-CSF (10 ng/ml) (R&D Systems, Minneapolis, MN).

Human embryonic kidney (HEK)-293 cells grown to confluence in DMEM supplemented with 10% heat-inactivated FCS, penicillin, streptomycin, 2 mM glutamine, and 10 mM Hepes (pH 7.4) were transfected with plasmid DNA encoding CCR5 (12). CD4-positive human osteosarcoma (HOS-CD4) cell lines transfected with individual chemokine receptor cDNAs were obtained from N. Landau, and were grown in the above culture medium supplemented with puromycin.

The derivative of the PM1 cell line chronically infected with the recombinant HIV-1 clone MV3-HXB2 has been described previously (11). sCD26 cleavage and electrospray mass spectrometry (ES-MS). To create the recombinant soluble human CD26 (sCD26) construct, a signal peptidase cleavage consensus sequence was introduced in the pTZ-CD26.11 cDNA (13) by a Leu to Ala substitution at residue 28. To obtain enzyme negative construct, the Ser at residue 630 was further replaced by Ala. The two constructs were cloned into the pEE14.HCMV expression vector and transfected into CHO-K1 cells (14). The enzymatically active (E+) and enzymatically deficient (E-) sCD26 proteins were purified from cell culture supernatants of stable transfectants, and were tested in Western blotting and DPPIV enzyme assays (15). Both proteins had a relative molecular weight of 110 kDa, bound equally well to several CD26 mAbs, but only the E+ sCD26 showed detectable DPPIV activity. rhRANTES, MCP-1, MCP-2, eotaxin, and IP-10 (100 nM) (Peprotech, Rocky Hill, NJ) were incubated overnight at 37oC with different amounts of E+ or E(sCD26 in 50 (1 of PBS. Samples were desalted and concentrated by using a peptide trap (Michrom BioResources, Inc., Auburn, CA), or a reversed-phase (RP) HPLC interface.

dose unit or else several smaller dose units and also by multiple administration of subdivided doses at specific intervals.

The pharmaceutical compositions according to the invention are in general administered topically, intravenously, orally or parenterally or as implants, but even rectal use is possible in principle. Suitable solid or liquid pharmaceutical preparation forms are, for example, granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, aerosols, drops or injectable solution in ampule form and also preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of present methods for drug delivery, see Langer, *Science*, 249: 1527-1533 (1990), which is incorporated herein by reference.

The pharmaceutical compositions according to the invention may be administered locally or systemically. By "therapeutically effective dose" is meant the quantity of a compound according to the invention necessary to prevent, to cure or at least partially arrest the symptoms of the disease and its complications. Amounts effective for this use will, of course, depend on the severity of the disease and the weight and general state of the patient. Typically, dosages used *in vitro* may provide useful guidance in the amounts useful for *in situ* administration of the pharmaceutical composition, and animal models may be used to determine effective dosages for treatment of particular disorders. Various considerations are described, *e.g.*, in Gilman *et al.* (eds.) (1990) GOODMAN AND GILMAN'S: THE PHARMACOLOGICAL BASES OF THERAPEUTICS, 8th ed., Pergamon Press; and REMINGTON'S PHARMACEU-TICAL SCIENCES, 17th ed. (1990), Mack Publishing Co., Easton, Pa., each of which is herein incorporated by reference.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following examples are to be considered illustrative and thus are not limiting of the remainder of the disclosure in any way whatsoever.

-26-

Mack Publishing Co., 1405-1412, 1461-1487 (1975) and *The National Formulary XIV.*, 14th ed. Washington: American Pharmaceutical Association (1975), the contents of which are hereby incorporated by reference. The pH and exact concentration of the various components of the pharmaceutical composition are adjusted according to routine skills in the art. *See*

5 *Goodman and Gilman's The Pharmacological Basis for Therapeutics* (7th ed.).

In another embodiment, the invention relates to a method of treating a subject having an HIV-related disorder associated with expression of CCR5 including administering to an HIV-infected or susceptible cell of a subject a therapeutically effective dose of a pharmaceutical composition containing the compounds of the present invention and a pharmaceutically

10 acceptable carrier. "Administering" the pharmaceutical composition of the present invention may be accomplished by any means known to the skilled artisan. By "subject" is meant any mammal, preferably a human. Such a method can be performed *in vivo* or *ex vivo* for example. For example, a vector containing a nucleic acid sequence encoding SEQ ID NO:2 or another truncated chemokine can be utilized for introducing the composition into a cell of the subject.

15 In another embodiment, the invention provides a method of treating a subject having or at risk of having an HIV infection or disorder, comprising administering to the subject, a therapeutically effective amount of a polypeptide of SEQ ID NO:2, wherein the polypeptide inhibits cell-cell fusion in cells infected with HIV. This method is performed as discussed above.

20 In another embodiment, the invention provides a method of inhibiting membrane fusion between HIV and a target cell or between an HIV-infected cell and a CD4 positive uninfected cell comprising contacting the target or CD4 positive cell with a fusion-inhibiting effective amount of the polypeptide of SEQ ID NO:2.

The pharmaceutical compositions are preferably prepared and administered in dose

25 units. Solid dose units are tablets, capsules and suppositories. For treatment of a patient, depending on activity of the compound, manner of administration, nature and severity of the disorder, age and body weight of the patient, different daily doses are necessary. Under certain circumstances, however, higher or lower daily doses may be appropriate. The administration of the daily dose can be carried out both by single administration in the form of an individual

Methods of diagnosis of chemokine-associated disorders

In another embodiment, the invention provides a method for diagnosis and prognosis of chemokine-associated disorders. The method includes identifying the presence of a chemokine of interest from a specimen isolated from the subject; determining the amino-terminal sequence of the chemokine, wherein a full-length amino acid sequence is indicative of the presence of a wild-type chemokine polypeptide and a truncated amino-terminal sequence is indicative of the presence of a variant chemokine; and determining the concentration of wild-type chemokine as compared to variant chemokine, thereby providing a diagnosis of the subject. This method is also useful for prognosis of a subject, for example, a subject having AIDS and being treated with a particular therapeutic regimen. The amino-terminal sequence of the chemokine is determined, for example, by standard N-terminal sequencing, or by contacting the chemokine with an antibody which distinguishes wild-type from variant chemokine polypeptide, as described above. Use of monoclonal antibodies, for example, allows simple detection by ELISA or other methods. Specimens useful for such diagnosis include but are not limited to blood, sputum, urine, saliva, cerebrospinal fluid, and serum.

Pharmaceutical compositions

The invention also includes various pharmaceutical compositions that are useful for therapeutic applications as described herein. The pharmaceutical compositions according to the invention are prepared by bringing a polypeptide such as SEQ ID NO:2 (RANTES (3-68)) or a DPPIV, such as CD26, into a form suitable for administration to a subject using carriers, excipients and additives or auxiliaries. Frequently used carriers or auxiliaries include magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents, such as sterile water, alcohols, glycerol and polyhydric alcohols. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial, anti-oxidants, chelating agents and inert gases. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described, for instance, in *Remington's Pharmaceutical Sciences*, 15th ed. Easton:

Preferably, a chemokine useful for inhibition allergic or inflammatory reactions is a truncated eotaxin.

The use of a truncated chemokine in the method of the invention may inhibit or depress an immune or inflammatory response where desirable, such as in graft rejection responses after organ and tissue transplantations, or autoimmune disease. Some of the commonly performed transplantation surgery today includes organs and tissues such as kidneys, hearts, livers, skin, pancreatic islets and bone marrow. However, in situations where the donors and recipients are not genetically identical, graft rejections can still occur. Autoimmune disorders refer to a group of diseases that are caused by reactions of the immune system to self antigens leading to tissue destruction. These responses may be mediated by antibodies, auto-reactive T cells or both. Some important autoimmune diseases include diabetes, autoimmune thyroiditis, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosis, and myasthenia gravis. Other allergic or inflammatory responses are included in the method of the invention.

In another embodiment, the invention provides a method for accelerating angiogenesis or wound healing in a subject, comprising administering to the subject an effective amount of an inhibitor of dipeptidyl peptidase IV (DPPIV) enzyme activity or gene expression or a DPPIV-insensitive chemokine, such that chemokine processing is inhibited, thereby accelerating angiogenesis or wound healing. For example, new blood vessels are required for tissue repair and enhanced blood vessel growth may aid in improving circulation to ischemic limbs and heart tissue suffering from atherosclerotic disease, healing skin ulcers or other wounds, and establishing tissue grafts. Preferably, a chemokine useful for accelerating angiogenesis is a wild-type IP-10. Cleavage of IP-10 appears to inactivate the activity of IP-10, therefore it is desirable to inhibit cleavage of IP-10. Alternatively, it may be desirable to provide a variant IP-10 polypeptide which contains an amino acid substitution at position 2, such that neither proline nor alanine is present, which would result in a DPPIV-insensitive chemokine. However, such a variant must retain the activity of wild-type IP-10, e.g., a chemoattractant for NK cells.

-23-

Inhibition of DPPIV

In another embodiment, the invention provides a method for inhibiting dipeptidyl peptidase IV (DPPIV)-mediated chemokine processing comprising contacting DPPIV with an inhibiting effective amount of a compound which inhibits DPPIV expression or activity.

5   For example, the method includes inhibiting CD26 expression or activity. To determine whether the DPPIV activity or expression is inhibited, an assay to detect cleavage of a chemokine having an alanine or proline at position 2, or a Northern blot analysis, can be performed, respectively. Other standard methods can be used to detect inhition of gene expression or enzymatic activity. For example, incubation of CD26, RANTES and a compound

10 suspected of inhibiting CD26 activity, would result in wild-type RANTES, but little or no cleaved RANTES (or RANTES "variant").

Methods of use for inhibiting HIV-1 replication, allergic or inflammatory reactions, and angiogenesis

In another embodiment, the invention provides a method for inhibiting HIV-1

15 replication in a host cell susceptible to HIV-1 infection, comprising contacting the cell or the host with an effective amount of dipeptidyl peptidase IV (DPPIV) enzyme such that macrophage-derived chemokine (MDC) or RANTES is cleaved to produce truncated MDC. or RANTES, respectively, thereby providing antiviral activity and inhibiting HIV-1 replication. The present invention provides data demonstrating that cleaved RANTES blocks

20 HIV-1 infection (EXAMPLE 7). While not wanting to be bound to a particular theory, it is believed that the activity of MDC is increased upon cleavage. MDC suppresses HIV-1 replication, thus, it is desirable for AIDS patients, or individuals at risk of HIV-1 infection to have increased levels of cleaved MDC. Other chemokines may also be useful in the method of the invention fro inhibiting HIV-1 replication.

25 In yet another embodiment, the invention provides a method for inhibiting an allergic or inflammatory reaction in a subject, comprising administering to the subject an effective amount of dipeptidyl peptidase IV (DPPIV) enzyme such that a chemokine is cleaved to produce a truncated chemokine, thereby inhibiting an allergic or inflammatory reaction.

oligonucleotide (ASO) probe analysis (Conner, *et al., Proc. Natl. Acad. Sci. USA,* 80:278, 1983), oligonucleotide ligation assays (OLAs) (Landegren, *et al., Science,* 241:1077, 1988), and the like. Molecular techniques for DNA analysis have been reviewed (Landegren, *et al., Science,* 242:229-237, 1988).

Methods for producing variant chemokines

In another embodiment, the invention provides a method for producing a variant chemokine having an activity different from the activity of the wild-type chemokine, including contacting the wild-type chemokine with an N-terminal processing effective amount of dipeptidyl peptidase IV (DPPIV), thereby truncating the chemokine and producing a variant chemokine. The term "N-terminal processing effective amount" refers to that amount of a DPPIV that cleaves the amino terminus of a wild-type chemokine polypeptide to produce a chemokine lacking the first two amino terminal amino acids. For example, incubation of RANTES with an "N-terminal processing effective amount" of CD26 results in RANTES (3-68) which has different activity than wild type RANTES. Chemokines that contain amino acid motifs at the N-terminus include but are not limited to RANTES, MIP-1, IP-10, eotaxin, macrophage-derived chemokine (MDC) and MCP-2. Other chemokines known in the art can be assessed for sensitivity to cleavage by DPPIVs as described herein by determining the first two amino terminal amino acids.

Contacting the chemokine can be *in vitro* or *in vivo.* For example, a specimen isolated from a subject, such as a human, or a mixture or pure sample of chemokine, can be contacted with DPPIV *in vitro.* The contacting of the DPPIV and chemokine is deemed sufficient when cleavage of the chemokine has occurred. It may be desirable to only cleave a fraction of the total chemokine population, therefore, samples can be analyzed at various time of incubation to determine the optimal conditions for the desired concentration of wild-type versus truncated variant chemokine achieved.

The preferred chemokine illustrated herein is RANTES and the preferred DPPIV is CD26. Other chemokines and DPPIVs are also included in the method of the invention.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells. See, for example, Larrick et al., METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, VOL. 2, page 106 (1991).

Screen for compounds which modulate DDPPIV

In another embodiment, the invention provides a method for identifying a compound which modulates dipeptidyl peptidase IV (DPPIV)-mediated chemokine processing. The method includes: a) incubating components comprising the compound, DPPIV and a chemokine under conditions sufficient to allow the components to interact; and b) determining the N-terminal amino acid sequence of the chemokine before and after incubating in the presence of the compound. Compounds that inhibit DPPIV include peptides, peptidomimetics, polypeptides, chemical compounds and biologic agents. Preferably the DPPIV is CD26. If a compound inhibits the DPPIV or CD26 enzymatic activity, the chemokine will have an N-terminal amino acid sequence which corresponds to the wild-type polypeptide. Alternatively, if the compound stimulates DPPIV or CD26 enzymatic activity, the chemokine will have a truncated amino-terminal amino acid sequence. The amino acid sequence can be determined by standard N-terminal sequencing methods or by contacting the chemokine with a monoclonal antibody which distinguishes between wild-type and truncated or variant chemokine, for example.

Incubating includes conditions which allow contact between the test compound and the chemokine and a DPPIV. Contacting includes in solution and in solid phase, or in a cell. The test compound may optionally be a combinatorial library for screening a plurality of compounds. Compounds identified in the method of the invention can be further evaluated, detected, cloned, sequenced, and the like, either in solution or after binding to a solid support, by any method usually applied to the detection of a specific DNA sequence such as PCR, oligomer restriction (Saiki, et al., Bio/Technology, 3:1008-1012, 1985), allele-specific

methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab'

5   monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. patents No. 4,036,945 and No. 4,331,647, and references contained therein. These patents are hereby incorporated in their entireties by reference. *See also* Nisonhoff *et al.*, *Arch. Biochem. Biophys.* 89:230 (1960); Porter, *Biochem. J.* 73:119

10   (1959); Edelman *et al.*, METHODS IN ENZYMOLOGY, VOL. 1, page 422 (Academic Press 1967); and Coligan *et al.* at sections 2.8.1-2.8.10 and 2.10.1-2.10.4.

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the

15   antigen that is recognized by the intact antibody.

For example, Fv fragments comprise an association of V_H and V_L chains. This association may be noncovalent, as described in Inbar *et al.*, *Proc. Nat'l Acad. Sci. USA* 69:2659 (1972). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. *See, e.g.*, Sandhu,

20   *supra*. Preferably, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as *E. coli*. The recombinant host cells

25   synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by Whitlow *et al.*, METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, VOL. 2, page 97 (1991); Bird *et al.*, *Science* 242:423-426 (1988); Ladner *et al.*, U.S. patent No. 4,946,778; Pack *et al.*, *Bio/Technology* 11: 1271-77 (1993); and Sandhu, *supra*.

murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin variable domains are described, for example, by Orlandi et al., Proc. Nat'l Acad. Sci. USA 86:3833 (1989), which is hereby incorporated in its entirety by reference. Techniques for producing humanized monoclonal antibodies are described, for example, by Jones et al., Nature 321: 522 (1986); Riechmann et al., Nature 332: 323 (1988); Verhoeyen et al., Science 239: 1534 (1988); Carter et al., Proc. Nat'l Acad. Sci. USA 89: 4285 (1992); Sandhu, Crit. Rev. Biotech. 12: 437 (1992); and Singer et al., J. Immunol. 150: 2844 (1993), which are hereby incorporated by reference.

Antibodies of the invention also may be derived from human antibody fragments isolated from a combinatorial immunoglobulin library. See, for example, Barbas et al., METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, VOL. 2, page 119 (1991); Winter et al., Ann. Rev. Immunol. 12: 433 (1994), which are hereby incorporated by reference. Cloning and expression vectors that are useful for producing a human immunoglobulin phage library can be obtained, for example, from STRATAGENE Cloning Systems (La Jolla, CA).

In addition, antibodies of the present invention may be derived from a human monoclonal antibody. Such antibodies are obtained from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain loci are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green et al., Nature Genet. 7:13 (1994); Lonberg et al., Nature 368:856 (1994); and Taylor et al., Int. Immunol. 6:579 (1994), which are hereby incorporated by reference.

Antibody fragments of the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in E. coli of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional

purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. *See, e.g.*, Coligan *et al.*, sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes *et al.*, *Purification of Immunoglobulin G (IgG)*, in
5   METHODS IN MOLECULAR BIOLOGY, VOL. 10, pages 79-104 (Humana Press 1992).

Methods of *in vitro* and *in vivo* multiplication of monoclonal antibodies is well-known to those skilled in the art. Multiplication *in vitro* may be carried out in suitable culture media such as Dulbecco's Modified Eagle Medium or RPMI 1640 medium, optionally replenished by a mammalian serum such as fetal calf serum or trace elements and growth-sustaining
10   supplements such as normal mouse peritoneal exudate cells, spleen cells, bone marrow macrophages. Production *in vitro* provides relatively pure antibody preparations and allows scale-up to yield large amounts of the desired antibodies. Large scale hybridoma cultivation can be carried out by homogenous suspension culture in an airlift reactor, in a continuous stirrer reactor, or in immobilized or entrapped cell culture. Multiplication *in vivo* may be
15   carried out by injecting cell clones into mammals histocompatible with the parent cells, *e.g.*, osyngeneic mice, to cause growth of antibody-producing tumors. Optionally, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. After one to three weeks, the desired monoclonal antibody is recovered from the body fluid of the animal.

20   Therapeutic applications for antibodies disclosed herein are also part of the present invention. For example, antibodies of the present invention may also be derived from subhuman primate antibody. General techniques for raising therapeutically useful antibodies in baboons can be found, for example, in Goldenberg *et al.*, International Patent Publication WO 91/11465 (1991) and Losman *et al.*, *Int. J. Cancer* 46:310 (1990), which are hereby
25   incorporated by reference.

Alternatively, a therapeutically useful anti-RANTES (3-68) or other variant chemokine antibody may be derived from a "humanized" monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementarity determining regions from heavy and light variable chains of the mouse immunoglobulin into a human
30   variable domain, and then substituting human residues in the framework regions of the

encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein. (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

Antibodies that distinguish wild-type chemokine from truncated chemokine

The present invention also provides antibodies useful for distinguishing between wild-type and DPPIV-truncated chemokine polypeptides. Preferably, the antibodies are produced by using N-terminal peptides having about 8 or more amino acids. Therefore, antibodies produced will distinguish between a chemokine, such as RANTES, that contains N-terminal amino acids, and a chemokine that has been cleaved, for example by CD26. The preparation of polyclonal antibodies is well-known to those skilled in the art. See, for example, Green *et al.*, *Production of Polyclonal Antisera*, in IMMUNOCHEMICAL PROTOCOLS (Manson, ed.), pages 1-5 (Humana Press 1992); Coligan *et al.*, *Production of Polyclonal Antisera in Rabbits, Rats, Mice and Hamsters*, in CURRENT PROTOCOLS IN IMMUNOLOGY, section 2.4.1 (1992), which are hereby incorporated by reference.

The preparation of monoclonal antibodies likewise is conventional. See, for example, Kohler & Milstein, *Nature* 256:495 (1975); Coligan *et al.*, sections 2.5.1-2.6.7; and Harlow *et al.*, ANTIBODIES: A LABORATORY MANUAL, page 726 (Cold Spring Harbor Pub. 1988), which are hereby incorporated by reference. Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures. Monoclonal antibodies can be isolated and

expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the RANTES (3-68) or other variant chemokine genetic sequences. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg, et al., Gene, 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, J. Biol. Chem., 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter (e.g., T7, m etallothionein I, or polyhedrin promoters).

Polynucleotide sequences encoding RANTES (3-68) or other variant chemokine can be expressed in either prokaryote or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryote are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention.

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as E. coli, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the CaCl$_2$ method using procedures well known in the art. Alternatively, MgCl or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell if desired.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding the RANTES (3-68) or other variant chemokine of the invention, and a second foreign DNA molecule

inhibit production of RANTES (3-68) or other variant chemokine polypeptides. In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and introduced into a target RANTES (3-68) or other variant chemokine-producing cell. The use of antisense methods to inhibit the translation of genes is known in the art, and is described, e.g., in Marcus-Sakura (Anal. Biochem., 172:289, 1988).

In addition, ribozyme nucleotide sequences for RANTES (3-68) or other variant chemokine are included in the invention. Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences encoding these RNAs, molecules can be engineered to recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech (1988) J. Amer. Med. Assn. 260:3030). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

There are two basic types of ribozymes, tetrahymena-type (Hasselhoff (1988) Nature 334:585) and "hammerhead"-type. Tetrahymena-type ribozymes recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to tetrahymena-type ribozymes for inactivating a specific mRNA species, and 18-base recognition sequences are preferable to shorter recognition sequences.

DNA sequences encoding RANTES (3-68) or other variant chemokine can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

In the present invention, the RANTES (3-68) or other variant chemokine polynucleo-tide sequences may be inserted into a recombinant expression vector. The term "recombinant

cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA.

Of the three above-noted methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common. This is especially true when it is desirable to obtain the microbial expression of mammalian polypeptides due to the presence of introns.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired polypeptide product is known. When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, *et al., Nucl. Acid Res.*, 11:2325, 1983).

A cDNA expression library, such as lambda gt11, can be screened indirectly for RANTES (3-68) or other variant chemokine peptides having at least one epitope, using antibodies specific for RANTES (3-68) or other variant chemokine. Such antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of RANTES (3-68) or other variant chemokine cDNA.

The isolated polynucleotide sequences of the invention also include sequences complementary to the polynucleotides encoding RANTES (3-68) or other variant chemokine (antisense sequences). Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub *et al.*, Scientific American 262:40, 1990). The invention includes all antisense polynucleotides that

-13-

These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences, 2) polymerase chain reaction (PCR) on genomic DNA or cDNA using primers capable of annealing to the DNA sequence of interest, and 3) antibody screening of expression libraries to detect cloned DNA fragments with shared structural features.

Preferably the RANTES (3-68) or other variant chemokine polynucleotide of the invention is derived from a mammalian organism, and most preferably from a mouse, rat, or human. Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Oligonucleotide probes, which correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, *et al.,* *Nucl. Acid Res.,* 9:879, 1981; Maniatis, *et al., Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor, N.Y. 1989).

The development of specific DNA sequences encoding RANTES (3-68) or other variant chemokine can also be obtained by: 1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the polypeptide of interest; and 3) *in vitro* synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor

independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequences.

Specifically disclosed herein is a DNA sequence containing the RANTES polypeptide gene encoding RANTES truncated at positions 1 and 2. The polynucleotide encoding

5   RANTES (3-68) includes Figure 8 (SEQ ID NO:1), as well as nucleic acid sequences complementary to SEQ ID NO:1. A complementary sequence may include an antisense nucleotide. When the sequence is RNA, the deoxynucleotides A, G, C, and T of SEQ ID NO:1 are replaced by ribonucleotides A, G, C, and U, respectively. Also included in the invention are fragments of the above-described nucleic acid sequences that are at least 15

10   bases in length, which is sufficient to permit the fragment to selectively hybridize to DNA that encodes the protein of SEQ ID NO: 2 under physiological conditions or a close family member of RANTES. The term "selectively hybridize" refers to hybridization under moderately or highly stringent conditions which excludes non-related nucleotide sequences.

In nucleic acid hybridization reactions, the conditions used to achieve a particular

15   level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition (e.g., GC v. AT content), and nucleic acid type (e.g., RNA v. DNA) of the hybridizing regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

20   An example of progressively higher stringency conditions is as follows: 2 x SSC/0.1% SDS at about room temperature (hybridization conditions); 0.2 x SSC/0.1% SDS at about room temperature (low stringency conditions); 0.2 x SSC/0.1% SDS at about 42°C (moderate stringency conditions); and 0.1 x SSC at about 68°C (high stringency conditions). Washing can be carried out using only one of these conditions, e.g., high stringency

25   conditions, or each of the conditions can be used, e.g., for 10-15 minutes each, in the order listed above, repeating any or all of the steps listed. However, as mentioned above, optimal conditions will vary, depending on the particular hybridization reaction involved, and can be determined empirically.

DNA sequences of the invention can be obtained by several methods. For example,

30   the DNA can be isolated using hybridization techniques which are well known in the art.

-11-

The invention provides isolated polynucleotides encoding the RANTES (3-68) or other variant chemokine polypeptide. In one embodiment, the polynucleotide is the nucleotide sequence of SEQ ID NO:1. These polynucleotides include DNA, cDNA and RNA sequences which encode RANTES (3-68) or other variant chemokine. It is understood that all polynucleotides encoding all or a portion of RANTES (3-68) or other variant chemokine are also included herein, as long as they encode a polypeptide with RANTES (3-68) or other variant chemokine activity (e.g., does not bind to CCR1 but binds to CCR5). Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides. For example, RANTES (3-68) or other variant chemokine polynucleotide may be subjected to site-directed mutagenesis. The polynucleotide sequence for RANTES (3-68) or other variant chemokine also includes antisense sequences. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of RANTES (3-68) or other variant chemokine polypeptide encoded by the nucleotide sequence is functionally unchanged. Abbreviations for the amino acid residues are follows: A, Ala; C, Cys; D, Asp: E, Glu: F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

As used herein, "polynucleotide" also refers to a nucleic acid sequence of deoxyribonucleotides or ribonucleotides in the form of a separate fragment or a component of a larger construct. DNA encoding portions or all of the polypeptides of the invention can be assembled from cDNA fragments or from oligonucleotides that provide a synthetic gene which can be expressed in a recombinant transcriptional unit.

An isolated polynucleotide as described herein is a nucleic acid molecule that is separated in some way from sequences in the naturally occurring genome of an organism. Thus, the term "isolated polynucleotide" includes any nucleic acid molecules that are not naturally occurring. The term therefore includes, for example, a recombinant polynucleotide which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule

referring to an chemokine polypeptide, means a polypeptide that is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. A substantially pure RANTES (3-68) or other variant chemokine polypeptide is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, RANTES (3-68) or other variant chemokine polypeptide. A substantially pure RANTES (3-68) or other variant chemokine can be obtained, for example, by extraction from a natural source; by expression of a recombinant nucleic acid encoding a RANTES (3-68) or other variant chemokine polypeptide, or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

Minor modifications of the recombinant RANTES (3-68) or other variant chemokine primary amino acid sequence may result in proteins which have substantially equivalent activity as compared to the RANTES (3-68) or other variant chemokine polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the biological activity of RANTES (3-68) or other variant chemokine still exists. Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant molecule without significantly altering its biological activity. This can lead to the development of a smaller active molecule which would have broader utility.

The polynucleotide sequence encoding the RANTES (3-68) or other variant chemokine polypeptide of the invention includes the disclosed sequence and conservative variations thereof. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

chemokine receptor, but it retained the ability to stimulate CCR5 receptors and to inhibit the cytopathic effects of HIV-1. Our results indicate that CD26-mediated processing together with cell activation induced changes in receptor expression provide an integrated mechanism for differential cell recruitment and for the regulation of target cell specificity of RANTES, and possibly other chemokines.

Nucleotide and amino acid sequences of RANTES variant (3-68) or other chemokine variants

In a first embodiment, the invention provides a substantially purified RANTES variant polypeptide exemplified by the amino acid sequence of SEQ ID NO:2. The term "polypeptide" means any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation); and includes natural proteins as well as synthetic or recombinant polypeptides and peptides.

The term "substantially pure" as used herein refers to RANTES (3-68) or other variant chemokine which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify RANTES (3-68) or other variant chemokine using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacryl-amide gel. The purity of the RANTES (3-68) or other variant chemokine polypeptide can also be determined by amino-terminal amino acid sequence analysis. RANTES (3-68) or other variant chemokine polypeptide includes functional fragments of the polypeptide, as long as the activity of RANTES (3-68) or other variant chemokine remains. Such functional variants would include the N-terminus which is truncated as compared to the wild-type RANTES or other chemokine. The term "variant" as used herein refers to a polypeptide having substantially the same polypeptide sequence as the corresponding wild-type polypeptide, with minor amino acid variations. These amino acid variations result in a polypeptide having various additional and/or different functions from the wild-type polypeptide, and possibly having altered receptor specificity as compared to the wild-type polypeptide. Smaller peptides containing the biological activity of RANTES (3-68) or other variant chemokine are included in the invention. The term "substantially pure," when

Figure 7. Effects of full-length and truncated RANTES on HIV-1-induced cytopathicity. (A) HOS-CD4.CCR5 cells were incubated with uninfected PM1 cells or PM1 cells chronically infected with MV3-HXB2 virus in the presence or absence of the indicated concentrations of RANTES variants. After 3 days, cell viability was measured by the XTT method. Data are means of triplicate samples (SEM, <20% of mean). (B) Representative photomicrographs of HOS-CD4.CCR5 cells cultured with HIV-1-infected PM1 cells in the absence or presence of RANTES (1-68) or RANTES(3-68) as indicated.

Figure 8. The nucleotide and deduced amino acid sequences for RANTES 3-68 (SEQ ID NO:1 and 2, respectively) are shown.

Description of the Preferred Embodiments

The present invention is based on the discovery of variant forms of chemokines which have different functions than their wild-type counterparts. These variant chemokines are produced by cleavage with a dipeptidyl peptidase IV (DPPIV) which cleaves at the N-terminus of a polypeptide when there is a proline or an alanine at position 2.

Overview

CD26 is a leukocyte activation marker that possesses dipeptidyl peptidase IV (DPPIV) activity but whose natural substrates and immunological functions have not been clearly defined. Several chemokines, including RANTES (regulated on activation, normal T expressed and secreted) have now been shown to be substrates for recombinant soluble human CD26 (sCD26). The truncated RANTES(3-68) lacked the ability of native RANTES(1-68) to increase the cytosolic calcium concentration in human monocytes, but it still induced this response in macrophages activated with macrophage colony-stimulating factor (M-CSF). Analysis of chemokine receptor messenger RNAs and patterns of desensitization of chemokine responses showed that the differential activity of the truncated molecule results from an altered receptor specificity. RANTES(3-68) showed a reduced activity, relative to that of RANTES(1-68), with cells expressing the recombinant CCR1

Figure 4. Effects of chemokines on [Ca2+]i in monocytes cultured in the absence (M) or presence (M + M-CSF) of M-CSF. Fura-2 labeled cells were exposed (at the times indicated by arrowheads) to chemically synthesized RANTES variants (100 nM) or other indicated rh chemokines (30 nM) (R & D Systems), and Ca2+ responses were measured. The final
5   concentrations of chemokines in this and subsequent experiments were sufficient to induce a maximal increase in [Ca2+]i in the responding cells, and further challenge with the same dose produced little or no detectable change in [Ca2+]i. The duration (~100 s) and amplitude (~20 to 30% of Fura-2 saturation) of Ca2+ responses were similar to those obtained for chemokines with human monocytes (36). Similar results were obtained in two additional
10   experiments.

Figure 5. Desensitization of chemokine-induced Ca2+ responses by full-length or truncated RANTES. Fura-2 labeled cells were stimulated first with 100 nM RANTES(1-68) or RANTES(3-68), or were left unstimulated. After ~150 s, the cells were challenged with the RANTES variants (100 nM) or other chemokines (30 nM) as indicated, and Ca2+ responses
15   were measured.

Figure 6. Activity of full-length and truncated RANTES in cells expressing recombinant CCR5 or CCR1 receptors. The [Ca2+]i was measured in HEK-293 cells expressing CCR5 (A and C) and HOS-CD4 cells expressing CCR1 (B and D). (A and B) Cells were stimulated with various concentrations of the two RANTES variants as indicated and
20   maximal fluorescence values were calculated from the peaks of the Ca2+ response curves. (C and D) Homologous and heterologous desensitization of the responses induced by RANTES(1-68) and RANTES(3-68) was measured in transfectants as described in Fig. 5.

identifying the presence of a chemokine of interest from a specimen isolated from the subject; determining the amino-terminal sequence of the chemokine,   wherein a full-length amino acid sequence is indicative of the presence of a wild-type chemokine polypeptide and a truncated amino-terminal sequence is indicative of the presence of a variant chemokine; and

5   determining the concentration of wild-type chemokine as compared to variant chemokine, thereby providing a diagnosis of the subject.

Brief Description of the Drawings

Figure 1. RANTES cleavage products after digestion with sCD26. RANTES was incubated overnight with the indicated amounts of enzymatically active (E+) or enzymatically deficient

10   (E() sCD26 and samples were subjected to ES-MS analysis. The peaks in the spectrum at masses of 7905 to 7906 and 7887 to 7890 are tentatively identified as [M+K+]+ of RANTES with (7904 daltons) and without (7886 daltons) a molecule of H_2O, respectively; the labeled peaks at the left of the spectrum correspond to each of these molecular ions minus a Ser-Pro dipeptide (184 daltons).

15   Figure 2. Competitive inhibition of DPPIV by RANTES(1-68). Colorimetric DPPIV enzyme assay was performed using human placental DPPIV and the Gly-Pro-pNA substrate, in the presence or absence of the test competitors Ile-Pro-Ile, RANTES(1-68), or RANTES(3-68); the competitor concentration is indicated on the horizontal axis. Data are means $\pm$ SEM (n = 3), except for the highest concentration of RANTES(1-68) and RANTES(3-68), for which

20   only one sample was assayed in order to conserve material. Similar results were obtained in a repeat experiment.

Figure 3. RT-PCR analysis of chemokine receptor transcripts in monocytes cultured in the absence (M) or presence (M + M-CSF) of M-CSF. Total cellular RNA was subjected to RT-PCR analysis as described in Materials and Methods. Control reactions performed without

25   reverse transcriptase were negative for each PCR product.

dipeptidyl peptidase IV (DPPIV), thereby truncating the chemokine and producing a variant chemokine. Chemokines may include, but are not limited to, RANTES, MIP-1, IP-10, eotaxin, MDC, and MCP-2.

The invention also provides a method for inhibiting HIV-1 replication in a host cell susceptible to HIV-1 infection, comprising contacting the cell or the host with an effective amount of dipeptidyl peptidase IV (DPPIV) enzyme such that macrophage-derived chemokine (MDC) is cleaved to produce truncated MDC, thereby providing antiviral activity and inhibiting HIV-1 replication and a A method for inhibiting HIV-1 replication in a host cell susceptible to HIV-1 infection, comprising contacting the cell or the host with an effective amount of dipeptidyl peptidase IV (DPPIV) enzyme such that RANTES is cleaved to produce truncated RANTES, thereby providing antiviral activity and inhibiting HIV-1 replication.

In another embodiment, the invention provides a method for inhibiting dipeptidyl peptidase IV (DPPIV)-mediated chemokine processing comprising contacting DPPIV with an inhibiting effective amount of a compound which inhibits DPPIV expression or activity.

In another embodiment, the invention provides a method for inhibiting an allergic or inflammatory reaction in a subject, comprising administering to the subject an effective amount of Dipeptidyl peptidase IV (DPPIV) enzyme such that a chemokine is cleaved to produce a truncated chemokine, thereby inhibiting an allergic or inflammatory reaction. Preferably, the chemokine is eotaxin.

In another embodiment, the invention provides a method for accelerating angiogenesis or wound healing in a subject, comprising administering to the subject an effective amount of an inhibitor of dipeptidyl peptidase IV (DPPIV) enzyme activity or gene expression or a DPPIV-insensitive chemokine, such that chemokine processing is inhibited, thereby accelerating angiogenesis or wound healing. One exemplary chemokine useful in the method for accelerating angiogenesis is IP-10.

In all of the above methods, the exemplary DPPIV shown in the present invention is CD26.

In yet another embodiment, the invention provides a method for diagnosis or prognosis of a subject having a chemokine-associated disorder. The method includes

-4-

In a first embodiment, the invention provides the nucleotide and amino acid sequence of truncated RANTES (3-68), which is the same as the wild-type RANTES with a Serine/Proline truncation at positions 1 and 2 from the N-terminus, respectively.

In another embodiment, the invention provides a method for identifying a compound which modulates dipeptidyl peptidase IV (DPPIV)-mediated chemokine processing. The method includes a) incubating components comprising the compound, DPPIV and a chemokine under conditions sufficient to allow the components to interact; and b) determining the N-terminal amino acid sequence of the chemokine before and after incubating in the presence of the compound. Modulation of DPPIV-mediated chemokine processing may be inhibition or stimulation of processing, for example. Compounds which modulate such processing include peptides, peptidomimetics, and other small molecule compounds.

In another embodiment, the invention provides a method of inhibiting membrane fusion between HIV and a target cell or between an HIV-infected cell and a CD4 positive uninfected cell by contacting the target or CD4 positive cell with a fusion-inhibiting effective amount of the polypeptide of SEQ ID NO:2 (RANTES 3-68).

The invention also provides a method of treating a subject having or at risk of having an HIV infection or disorder, including administering to the subject, a therapeutically effective amount of a polypeptide of SEQ ID NO:2, wherein the polypeptide inhibits cell-cell fusion in cells infected with HIV. The invention also provides a method of treating a subject having an HIV-related disorder associated with expression of CCR5 comprising administering to an HIV infected or susceptible cell of the subject, a polypeptide of SEQ ID NO:2 or a nucleic acid sequence encoding the polypeptide of SEQ ID NO:2 or other variant chemokine. Preferably, the subject is a human.

Also included are pharmaceutical compositions including the polypeptide of SEQ ID NO:2 or CD26, in pharmaceutically acceptable carriers.

In yet another embodiment, the invention provides a method for producing a variant chemokine having an activity different from the activity of the wild-type chemokine, including contacting the wild-type chemokine with an N-terminal processing effective amount of

defined, but they may include regulation of the expression of chemokines and their receptors (2) as well as the modification of chemokine activity by posttranslational processing (3-5). Several chemokines share a conserved NH2-X-Pro sequence (X, any amino acid) at the NH2-terminus (6), which conforms to the substrate specificity of dipeptidyl exopeptidase IV (DPPIV) (7). DPPIV cleaves the first two amino acids from peptides with penultimate proline or alanine residues, although no natural substrate with immune function has been identified. This enzyme is also a leukocyte differentiation antigen, known as CD26 (8-10), that is expressed on the cell surface mostly by T lymphocytes and macrophages. Expression of CD26 has been associated with T cell activation (8-10) and with susceptibility of a T cell line to infection with macrophage-tropic (M-tropic) HIV-1 (11).

Summary of the Invention

The present invention is based on the discovery that chemokines having a particular N-terminal motif are natural substrates for a dipeptidyl dipeptidase (DPPIV). Prior to the present invention, it was known that CD26 is a leukocyte activation marker that possesses dipeptidyl peptidase IV (DPPIV) activity but natural substrates had not been identified. The present invention shows that several chemokines, including RANTES (regulated on activation, normal T expressed and secreted) are substrates for recombinant soluble human CD26 (sCD26). The present invention shows that DPPIV, *e.g.*, CD26-mediated processing, together with cell activation induces changes in receptor expression and provides a mechanism for differential cell recruitment and for the regulation of target cell specificity of chemokines.

Abbreviations: $[Ca2+]i$, cytosolic free Ca2+ concentration; DPPIV, dipeptidyl peptidase IV; ES-MS, electrospray mass spectrometry; M-tropic, macrophage-tropic; pNA, p-nitroanilide; rh, recombinant human; sCD26, soluble CD26.

CHEMOKINE VARIANTS AND METHODS OF USE

Field of the Invention

5   The present invention relates generally to chemoattractant cytokines, called chemokines, and more specifically to truncated or variant forms of chemokines which have functions different from their wild-type counterparts, methods of use and methods of producing such variant chemokines.

Background of the Invention

10   Immunomodulatory proteins include chemotactic cytokines, called "chemokines". Chemokines are small molecular weight immune ligands which are chemoattractants for leukocytes, such as especially neutrophils, basophils, monocytes and T cells. There are two major classes of chemokines which both contain four conserved cysteine residues which form disulfide bonds in the tertiary structure of the proteins. The α class is designated C-X-C
15   (where X is any amino acid), which includes IL-8, CTAP-III, gro/MGSA and ENA-78; and the β class, designated C-C, which includes MCP-1, MIP-1α and β, and regulated on activation, normal T expressed and secreted protein (RANTES). The designations of the classes are according to whether an intervening residue spaces the first two cysteines in the motif. In general, most C-X-C chemokines are chemoattractants for neutrophils but not
20   monocytes, whereas C-C chemokines appear to attract monocytes but not neutrophils. Recently, a third group of chemokines, the "C" group, was designated by the discovery of a new protein called lymphotactin (Kelner, et al., Science, 266:1395-1933, 1994). The chemokine family is believed to be critically important in the infiltration of lymphocytes and monocytes into sites of inflammation.

25   Monocytes differentiate into macrophages as they migrate from the blood to tissues during immune surveillance. At sites of inflammation, monocyte infiltration and macrophage accumulation are coordinated, in part, by chemokines (1). The mechanisms that control the recruitment of monocytes and macrophages by chemoattractants have not been clearly

(51) International Patent Classification 6 : **C12N 15/19, A61K 38/19, 38/48, C07K 16/24, G01N 33/68, C07K 14/52, C12N 5/10**	A2	(11) International Publication Number: **WO 99/28474**
		(43) International Publication Date: 10 June 1999 (10.06.99)

(54) Title: CHEMOKINE VARIANTS AND METHODS OF USE

(57) Abstract

The present invention provides the nucleotide and amino acid sequence of truncated RANTES (3–68), which has the same amino acid sequence as the wild–type RANTES, but with a Serine/Proline truncation at positions 1 and 2 from the N–terminus, respectively. CD26 is a leukocyte activation marker that possesses dipeptidyl peptidase IV (DPPIV) activity but whose natural substrates and immunological functions had not been previously defined. Several chemokines, including RANTES (regulated on activation, normal T expressed and secreted) are provided, which are substrates for human CD26. The truncated RANTES (3–68) lacked the ability of native RANTES (1–68) to increase the cytosolic calcium concentration in human monocytes, but it still induces this response in macrophages activated with macrophage colony–stimulating factor (M–CSF). RANTES (3–68) retains the ability of stimulate CCR5 receptors and to inhibit the cytopathic effects of HIV–1. The invention provides methods for identifying compounds that affect DPPIV–mediated chemokine cleavage, methods for inhibiting HIV infection and treating individuals having or at risk of having HIV infection, methods for diagnosis and/or prognosis of individuals having a chemokine–associated disorder and methods for accelerating wound healing and angiogenesis, all based on the discovery of DPPIV–mediated cleavage of chemokines.
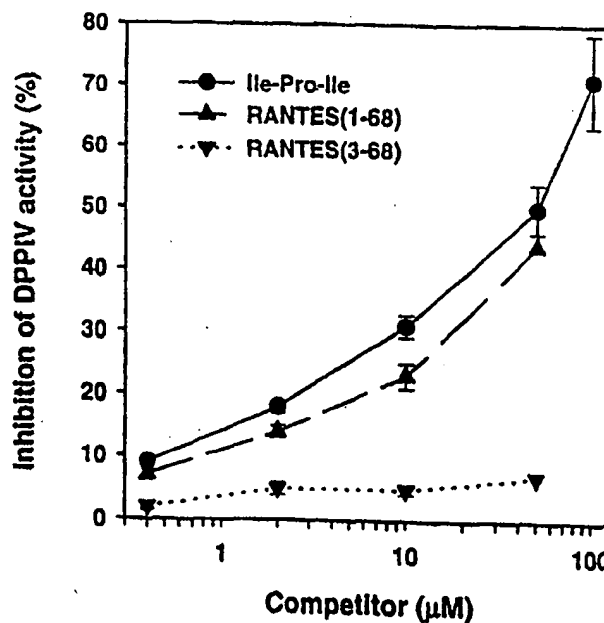
A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 38/00, 31/675; A01N 57/00; C07F 9/02, 9/547, 9/28, 9/06, 9/22; C07D 223/00, 225/00, 295/00, 279/04, 279/06, 279/10, 279/12, 265/04, 265/30

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/87, 89, 94, 19; 548/412, 111; 546/21; 540/542, 450; 544/53, 57, 88, 106; 558/170